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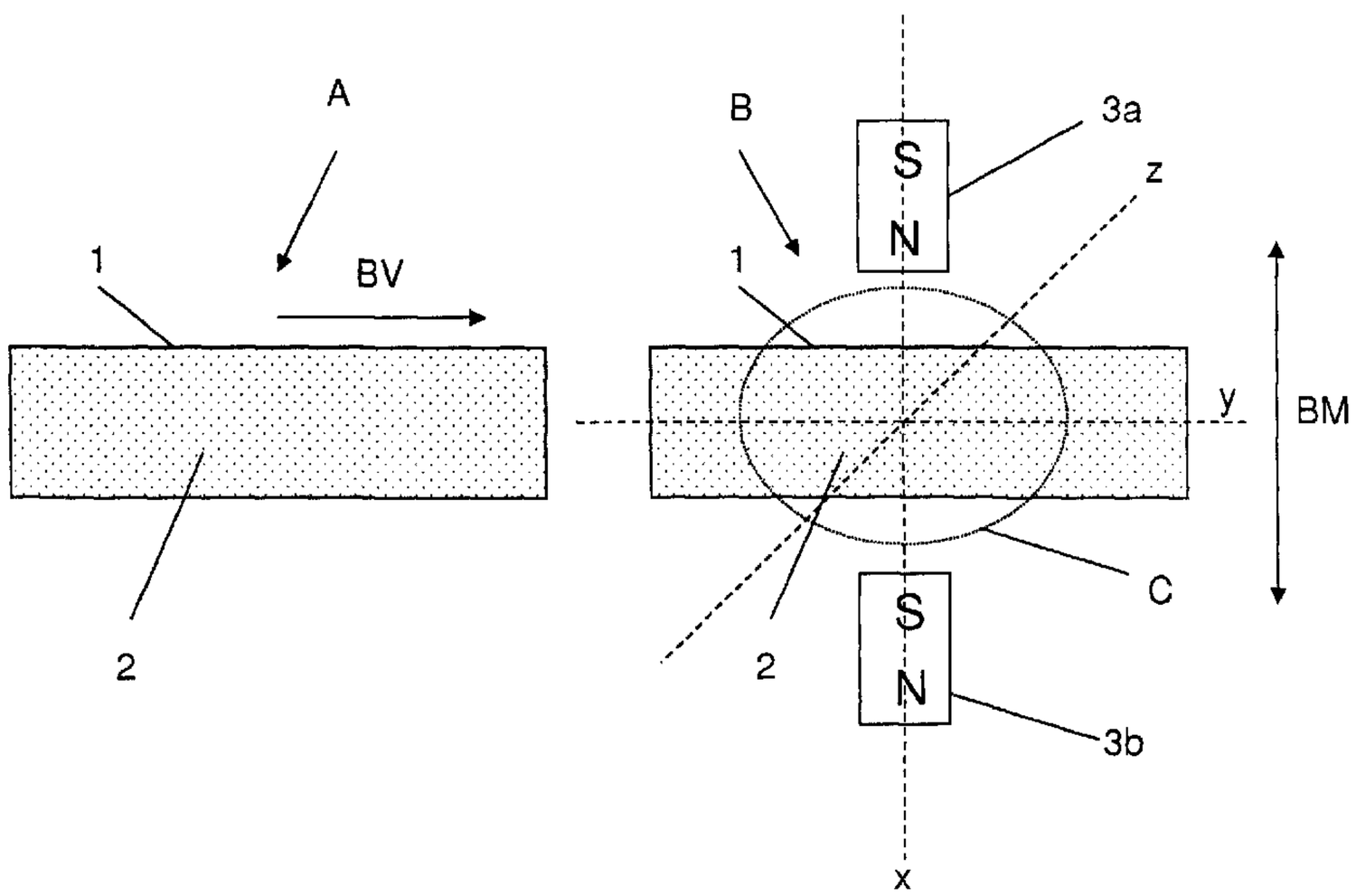
(72) Inventeurs/Inventors:
KERN, PETER, DE;
BACKES, HERBERT, DE

(73) Propriétaire/Owner:
AIRBUS DS GMBH, DE

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : PROCEDE D'EXECUTION D'ESSAIS IMMUNOLOGIQUES EN APESANTEUR
(54) Title: METHOD FOR PERFORMING IMMUNOASSAYS UNDER WEIGHTLESSNESS



(57) Abrégé/Abstract:
The invention relates to a method for controlled movement of magnetic carriers (2) in a sample volume (1) for performing immunoassays under weightless or reduced-weight conditions, wherein the magnetic carriers (2) are moved inside the sample volume (1) by means of permanent magnets (3a, 3b) movably arranged relative to at least one spatial axis (x, y, z) of the sample volume (1).

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(71) Anmelder: **ASTRIUM GMBH** [DE/DE]; Robert-Koch-Str. 1, 82024 Taufkirchen (DE).

(72) Erfinder: **KERN, Peter**; Aachstr. 3/2, 88626 Salem (DE). **BACKES, Herbert**; Im Königsfeld 66b, 66130 Saarbrücken (DE).

(74) Anwalt: **DAUB, Thomas**; Bahnhofstraße 5, 88662 Überlingen (DE).

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[Fortsetzung auf der nächsten Seite]

(54) **Title**: METHOD FOR PERFORMING IMMUNOASSAYS UNDER WEIGHTLESS CONDITIONS

(54) **Bezeichnung** : VERFAHREN ZUR DURCHFÜHRUNG VON IMMUNO-ASSAYS IN SCHWERELOSIGKEIT

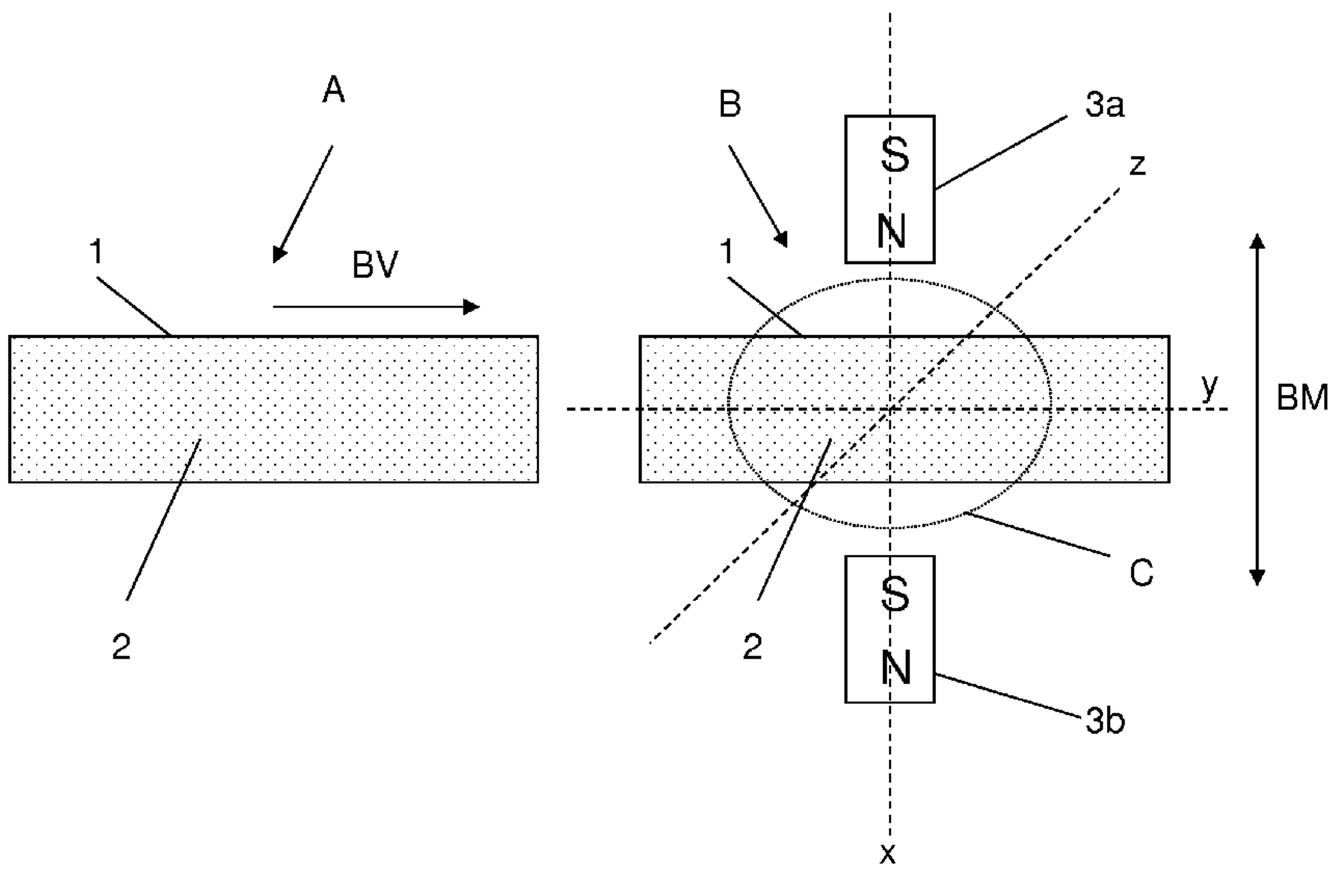

Fig. 1

(57) **Abstract**: The invention relates to a method for controlled movement of magnetic carriers (2) in a sample volume (1) for performing immunoassays under weightless or reduced-weight conditions, wherein the magnetic carriers (2) are moved inside the sample volume (1) by means of permanent magnets (3a, 3b) movably arranged relative to at least one spatial axis (x, y, z) of the sample volume (1).

(57) **Zusammenfassung**:

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Veröffentlicht:

— *mit internationalem Recherchenbericht (Artikel 21 Absatz 3)*

Die Erfindung betrifft ein Verfahren zum kontrollierten Bewegen von magnetischen Trägern (2) in einem Probenvolumen (1) zur Durchführung von Immuno-Assays in Schwerelosigkeit oder reduzierter Schwerkraft, wobei die magnetischen Träger (2) innerhalb des Probenvolumens (1) mittels bezüglich wenigstens einer Raumachse (x, y, z) des Probenvolumens (1) verschiebbar angeordneter Permanentmagnete (3a, 3b) bewegt werden.

Method for Performing Immunoassays Under Weightlessness

The invention relates to a method for moving magnetic carriers in a controlled manner in a sample volume for performing immunoassays.

In biochemical analysis, the use of immunoassays is widespread. This method allows selective quantitative or qualitative determination of single (monoplex) or several (multiplex) analytical parameters in a mostly complex biological matrix, such as for example blood, plasma, serum, urine, saliva, tears, sweat, culture media, cell extracts, cell suspensions, etc., which can contain a large number of substances.

The general principle of immunoassays is that the desired analyte selectively binds to a specific protein-based capture antibody or to specific DNA, RNA or functional subgroups or segments based thereon (capture antibody = cAB) and is labeled by a detection antibody (detection antibody = dAB). The cAB is mostly situated on a stationary carrier (solid phase).

In the standard literature, the nomenclature of the term "immunoassay" is inconsistent. Below, both for classical immunoassays, and also ELISA (ELISA = enzyme linked immunosorbent assay) with the use of enzymes, the term "immunoassay" is understood to mean that:

a) in classical immunoassays, the dAB carries either a dye or a fluorophor, which are detected by spectrometry or fluorimetry

b) the ELISAs (ELISA = enzyme linked immunosorbent assay) are a further immunoassay modification. These use an enzyme bound to dAB as the functional label element. Since the start of the 1980s, the ELISAs have replaced the RIAs (Radio-Immuno Assays) which used a radioisotope as the label. The enzyme bound to the analyte-antibody complex via the dAB converts an added enzyme-specific substrate into a detectable substance which can be detected in the solution by spectrometry or fluorimetry or by means of another physical effect, e.g. chemiluminescence.

In terrestrial use, the various solutions/substance are added sequentially. The free, non-bound substances/reactants are removed by washing steps. The complexes formed

remain because of their binding to the stationary phase in the reaction vessel, where they can then be detected.

Mobile carriers are a special form of the solid phase. These are so-called beads (diameter: nm - mm, but mostly a few µm), onto the surface whereof the cAB molecules are bound. After the washing step, these carriers are separated from the supernatant or the residual solution by centrifugation or in the case of magnetic carriers by means of strong magnets. After completion of the overall reaction of the immunoassay, in terrestrial applications the labeled carriers are read off either in a flow cytometer, a reading device for multiwell plates or an array reader. This can be effected as an integral measurement value or by image processing for each individual carrier or each array spot.

The steps described apply for immunoassays as a sandwich assay, as a competitive assay or also in the form of an ELISA.

Immunoassays are also to be used in space flights under reduced gravity, or even weightlessness (µg). This means that substance transport or substance separation are slowed or entirely prevented because of the reduced or absent gravity. During sample preparation on Earth, the reaction partners are moved in special mechanical mixers (e.g. orbital mixers or orbital shakers). Sedimentation for the observation occurs by means of gravity.

Immunoassays with magnetic carriers are widespread for use on Earth below 1µg. Previously, however, the magnetic carriers were primarily used for separation during a washing step. The terrestrial procedures for immunoassays, for cell concentration or separation are not suitable for use in space.

The objective of the invention is to provide a process with which the implementation of immunoassays with magnetic carriers is possible under weightlessness or reduced gravity.

According to the invention, for moving magnetic carriers in a controlled manner in a sample volume for performing immunoassays under weightlessness, the magnetic

carriers within the sample volume are moved by means of permanent magnets slidably arranged relative to at least one spatial axis of the sample volume and for mixing of the magnetic carriers the permanent magnets arranged on one spatial axis are moved in phase.

The use of magnetic carriers e.g. as a solid phase enables active, controlled, convective mixing of the reaction partners by external magnetic fields which for example operate sequentially from different directions. In addition, the substance transport is improved and the reaction rate increased. A further advantage is that the procedure becomes reproducible under weightlessness.

Finally, planar positioning of the magnetic particles for the purpose of detection (e.g. in the focal point of a microscope) is possible through a directed magnetic field which can be deliberately activated at a predetermined time.

Furthermore, it is possible to collect or hold the magnetic carriers in a defined region, e.g. during a change of fluid or a washing process, by means of a directed magnetic field which can be deliberately activated.

In addition, the magnetic carriers which are coated with a cAB can also be used for binding to specific cell types or membrane receptors, and in space experiments with reduced gravity these can be separated or concentrated or supplied by mechanical displacement for detection.

The absent or reduced gravity during the use of immunoassays in space is compensated by the appropriate use of magnetic carriers. The magnetic carriers are influenced by external magnetic fields activated in a controlled manner depending on the process step.

For mixing of magnetic carriers in a sample volume, the permanent magnets are advantageously arranged diametrically opposite relative to the sample volume.

For positioning of magnetic carriers on one plane within the sample volume, permanent magnets on a spatial axis that is perpendicular to the plane, where the permanent magnets lie, relative to the plane, diametrically opposite the magnetic carriers to be positioned, are advantageously in a first step moved in the direction of the sample volume and in a second step moved away from the sample volume.

The invention and further advantageous embodiments of the invention are explained in more detail below on the basis of diagrams:

Fig. 1 shows an example of a schematic arrangement for performing the method according to the invention in a first application,

Fig. 2 shows an example of a schematic arrangement for performing the method according to the invention in a second application, and

Fig. 3 shows an example of an implementation of a permanent magnet.

Fig. 1 shows an example of a schematic arrangement for mixing magnetic carriers 2 within a sample volume 1. Outside the sample volume 1, permanent magnets 3a and 3b are arranged on one spatial axis x,y,z of the sample volume 1. For clearer representation, only 2 permanent magnets 3 on the spatial axis x are shown in Fig. 1. Of course, further permanent magnets 3a and 3b can be arranged on the other spatial axes y and z.

The two permanent magnets 3a and 3b are arranged diametrically opposite relative to the sample volume 1, i.e. the sample volume 1 can be introduced into a region C between the two permanent magnets 3a and 3b. As is well-known, each permanent magnet 3a and 3b consists of a north pole N and a south pole S. It is advisable that the two permanent magnet 3a and 3b are arranged so that in each case the north and south pole are facing.

Fig. 1 shows the arrangement with the sample volume 1 in a first position A, in which the sample volume 1 is situated outside the region B between the two permanent magnets 3a and 3b. The sample volume 1 can be shifted according to the arrow direction BV into a position B, so that the sample volume 1 is situated in the region C. Of course, it is also possible that the two permanent magnets 3a and 3b are appropriately shifted.

For mixing of the magnetic carriers 2 in the sample volume 1, the sample volume 1 is brought into position B. Next, the two permanent magnets 3a and 3b are moved backwards and forwards in phase according to the arrow direction BM. The magnetic carriers 2 are now alternatingly oriented in the sample volume 1 in accordance with the adjacent magnetic field and correspondingly moved. Through the in-phase backward and forward movement of the two permanent magnets 3a and 3b, thorough mixing of the magnetic carriers 2 in the sample volume 1 is effected.

By appropriate arrangement and movement of other permanent magnets on the spatial axes y and z, the mixing can be improved.

Fig. 2 shows an example of a schematic arrangement for positioning magnetic carriers 2 within a sample volume 1. The diagram shows a sample in position B corresponding to Fig. 1. For positioning of magnetic carriers 2 on the plane 5, the permanent magnet 3a, described below as the positioning permanent magnet, which is arranged on an axis x that is perpendicular to the positioning plane 5, is used. This permanent magnet 3a which relative to the positioning plane lies diametrically opposite the magnetic carriers 2 to be positioned can be shifted in accordance with the arrow directions BM1, BM2.

Another permanent magnet 3b relative to the sample volume 1 arranged diametrically to the positioning permanent magnet 3a on the spatial axis x is shifted into a parking position P and protected by means of a screening device 4, so that magnetic fields of the permanent magnet 3b can have no influence on the magnetic carriers 2 in the sample volume 1.

For positioning the magnetic carriers 2 in the sample volume 1, the positioning permanent magnet 3a is shifted in the direction BM1 of the plane 5. Thereby, the magnetic carriers 2 are oriented and moved in the direction of the plane 5. Next, the positioning permanent magnet 3a is shifted in the direction BM2 and shifted into a corresponding parking position P (not shown).

During use in space, the magnetic carriers remain in this position until the end of the detection, since because of the reduced gravity no sedimentation or thermal convection occurs in the sample volume.

Fig. 3 shows by way of example the implementation of a permanent magnet. The permanent magnets are advantageously implemented as a matrix. The permanent magnet 3a comprises several permanent magnets 30a, which are advantageously arranged as a matrix wherein the permanent magnets 30a are arranged alternately.

Patent Claims

1. A method for moving magnetic carriers in a controlled manner in a sample volume for performing immunoassays under weightlessness or reduced gravity, wherein because of the reduced gravity no sedimentation and no convective heat transfer occurs in the sample volume and wherein the magnetic carriers are moved within the sample volume by means of permanent magnets slidably arranged relative to at least one spatial axis (x, y, z) of the sample volume, wherein to mix the magnetic carriers the permanent magnets arranged on one spatial axis (x, y, z) are moved in phase, wherein by the use of magnetic carriers active, controlled, convective mixing of reaction partners is effected by external magnetic fields, wherein during a change of fluid or a washing process, the magnetic carriers are collected or held by means of a directed magnetic field which can be deliberately activated, and wherein the magnetic carriers, for the purpose of detection are positioned planar by a directed magnetic field which can be deliberately activated, wherein the permanent magnets are shifted into a parking position (P) and protected by a screening device when not used in a process step, wherein the permanent magnets comprise several permanent magnets that are arranged as a matrix in which the permanent magnets are arranged alternately, wherein, for positioning of the magnetic carriers on a plane within the sample volume, the permanent magnets are moved on a spatial axis (x, y, z) that is perpendicular to the plane, wherein the permanent magnets lie, relative to the plane, diametrically opposite the magnetic carriers to be positioned, are in a first step moved in the direction of the sample volume and are in a second step moved away from the sample volume.

2. The method as claimed in claim 1, wherein the permanent magnets are arranged diametrically opposite relative to the sample volume.

3. The method as claimed in claim 1 or 2, wherein the permanent magnets are each arranged in the form of an array.

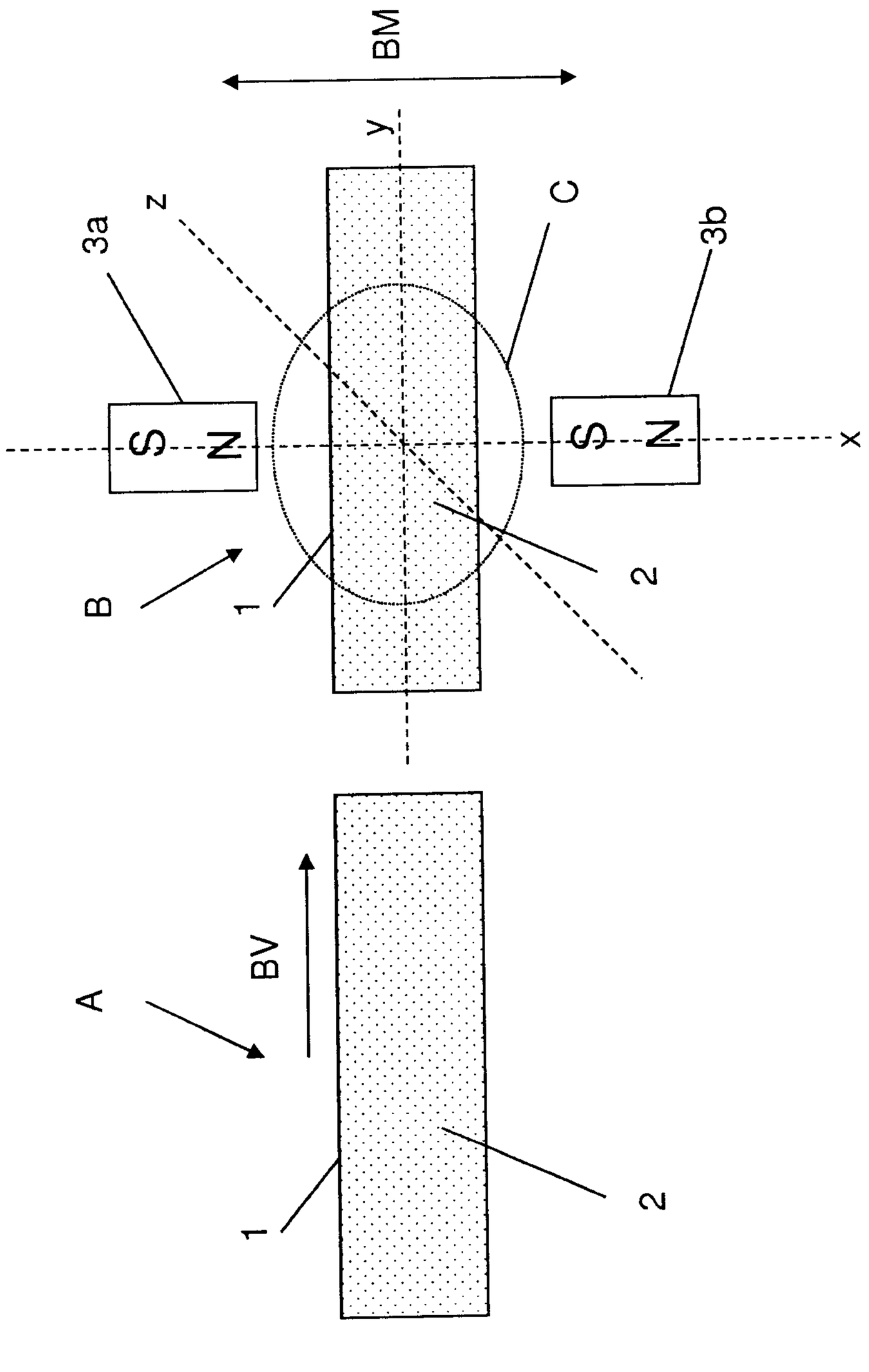


Fig. 1

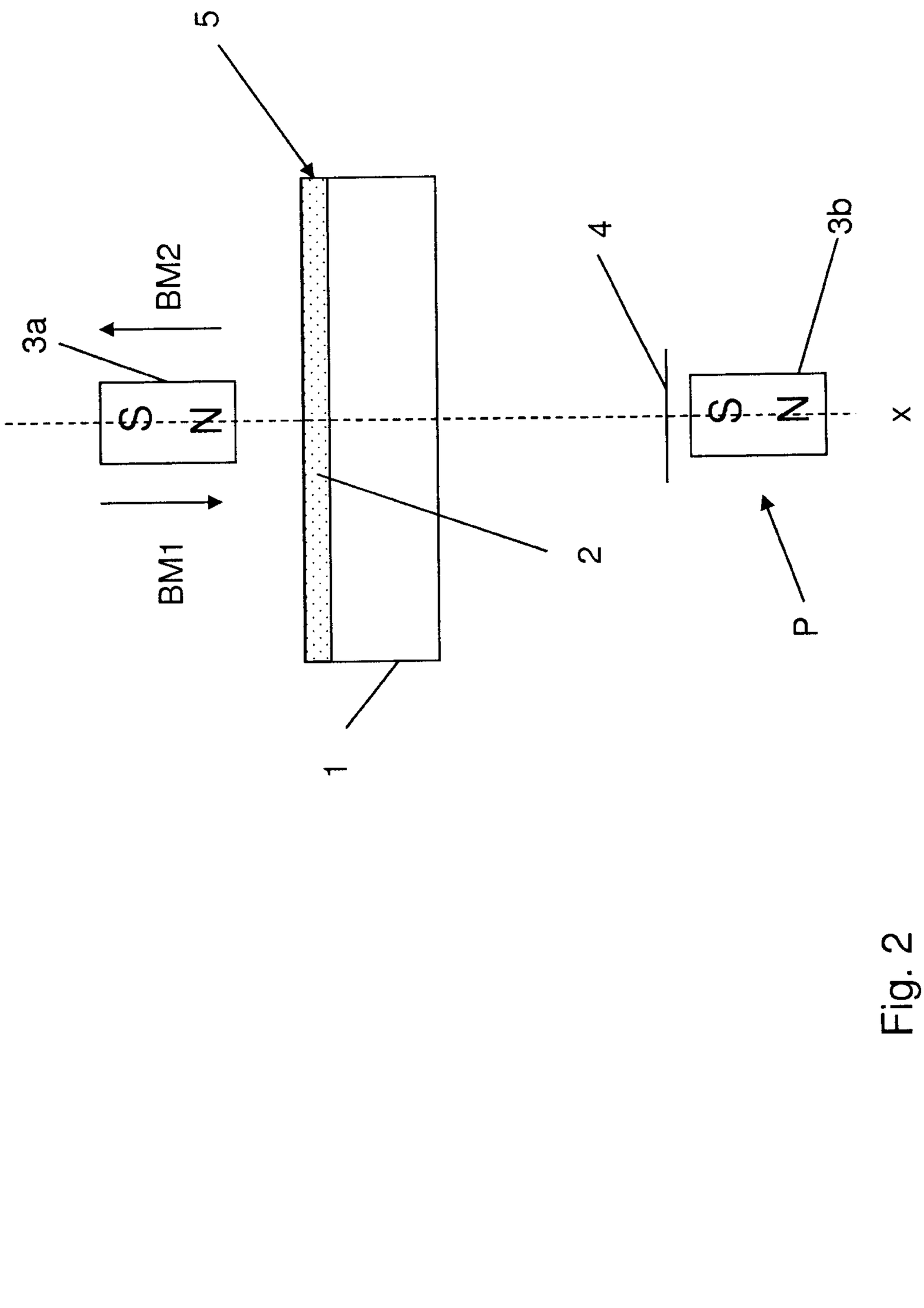


Fig. 2

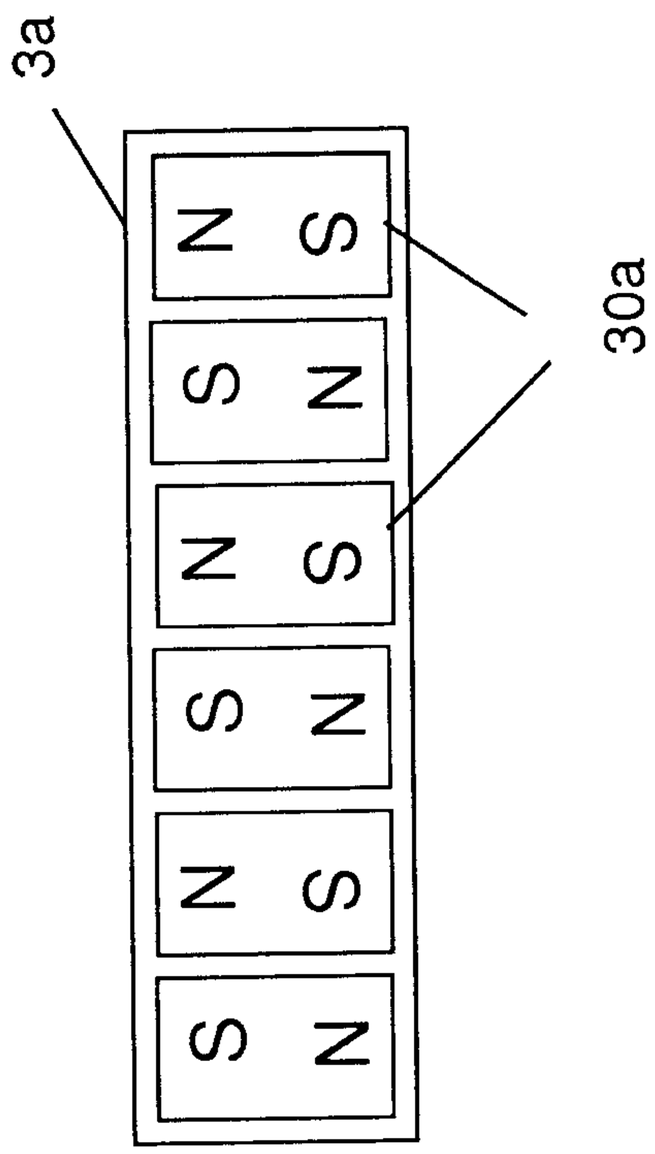
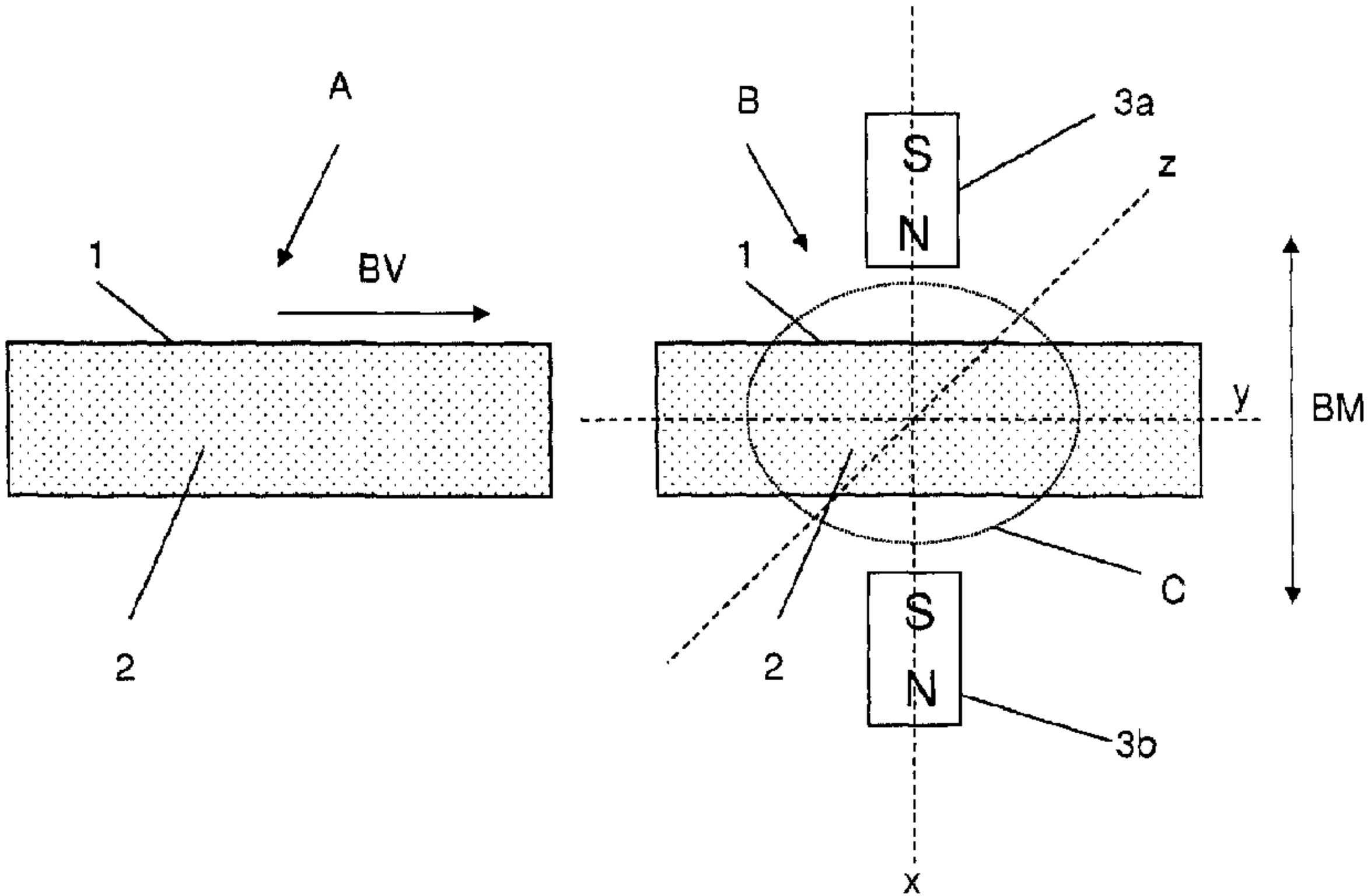


Fig. 3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