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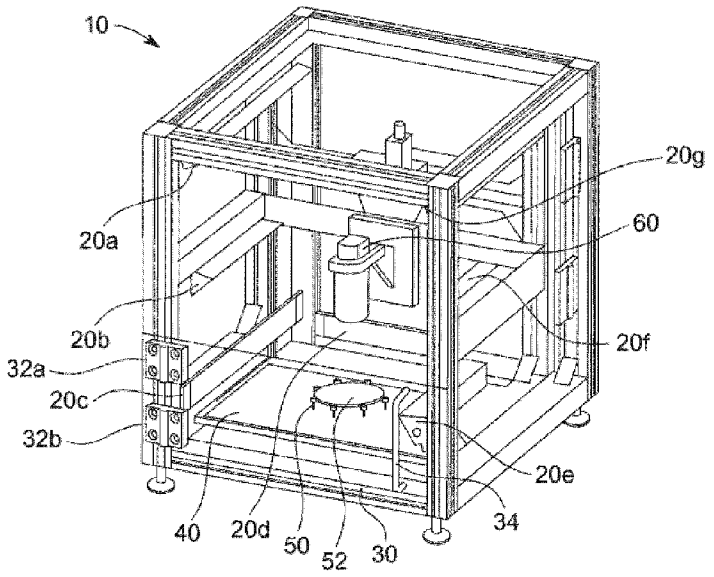

FIG. 1A

(57) Abstract: A method of illuminating a colony-forming unit sample method comprises: illuminating a transparent or translucent sample holder containing colony-forming units using at least one indirect illumination means and backlighting illumination means; capturing at least one image of the transparent or translucent sample holder when illuminated by said at least one indirect illumination means and said backlighting illumination means. In addition an apparatus for illuminating a colony-forming unit sample comprises: means configured to receive a transparent or translucent sample holder containing colony-forming units; at least one indirect illumination means configured to indirectly illuminate the transparent or translucent sample holder, a backlighting illumination means configured to backlight the transparent or translucent sample holder, and an image forming device positioned above said receiving means and configured to capture at least one image of the transparent or translucent sample holder.

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A Method and Apparatus for Illuminating Colony-Forming Units

Field of the Invention

This invention relates to method and apparatus for illuminating colony-forming unit samples.

Background to the Invention

Microbial Detection And Identification Applications

Microbial monitoring programs demonstrate that manufacturing environments are under control and ensure that products, manufacturing environments, and packaging are free from viable microbial contaminants. Microbial monitoring programs encompass environmental monitoring, bioburden testing and microbial identification. Environmental monitoring methods assess the air quality and surface hygiene of a manufacturing facility. Bioburden methods assess the microbial content on a product, a surface or with the environment. The test methods for conducting bioburden, include colony counting from pour plates, spread plates, and membrane filtration techniques. Microbial identification serves to provide an understanding the microbial ecology of a manufacturing facility, monitor the effectiveness of microbial control in aseptic environments and provide an investigative tool into excursions from normal microbial populations or sterility failures. Broad categorisation of microbial organisms by phenotypic identification is based on characteristics such as colony morphology, gram stain reactivity, microscopic morphology, growth temperature and biochemical testing outcomes.

The applications above rely on the counting of Colony-forming units (CFUs). Colony-forming units (CFUs) is a parameter which measures the number of viable (living) microorganisms such as bacteria or fungal cells in a sample.

CFU count plays an important role in Good Manufacturing Practice (GMP) environments and is imperative for regulatory approval of medical devices and pharmaceutical products. Colony counters measure the number of CFUs in a sample. Typically, this is performed by manual counting of CFUs in a sample.

Counting of CFUs (also termed colony numeration) generally requires culturing of the microorganism on a sample holder such as a culture plate containing a culture medium. An example would be a petri dish with agar solution as a growth medium for the microorganism. Such a sample holder is subjected to the optimal temperature in an incubator for a given period. The traditional method of CFU counting involves visible counting with the human eye using a clicker for tracking the count.

Summary of the Invention

The invention sets out a method of illuminating a colony-forming unit sample, the method comprising: illuminating a transparent or translucent sample holder containing colony-forming units using at least one indirect illumination means and backlighting illumination means;
capturing at least one image of the transparent or translucent sample holder when illuminated by said at least one indirect illumination means and said backlighting illumination means.

According an advantageous embodiment the method includes causing the backlighting illumination means to backlight said sample holder using uniform lighting in a single colour.

According a further advantageous embodiment the method includes causing the backlighting illumination means to backlight said sample holder using patterned lighting.
According a further advantageous embodiment the the patterned lighting comprises a striped pattern.

According a further advantageous embodiment the striped pattern comprises parallel stripes

According a further advantageous embodiment the the striped pattern comprises a plurality of spaced apart stripes on a background, the stripes being darker than the background.

According a further advantageous embodiment the the striped pattern comprises a plurality of stripes on a white background.

According a further advantageous embodiment the striped pattern comprises black stripes.

According a further advantageous embodiment the striped pattern comprises blue stripes

According a further advantageous embodiment the method includes capturing a plurality of images in sequence, and causing said backlighting illumination means to provide respective different backlighting illumination for each image, wherein, preferably, the plurality of images in sequence comprises patterned lighting and/or uniform lighting in a single colour or a combinations of both.

According a further advantageous embodiment the, or each, image is processed and wherein the processing comprises employing a counting algorithm which analyses the, or each, image to count any colony-forming units rendered visible by the illumination; and reporting or recording the number of colony-forming units.

According a further advantageous embodiment the counting algorithm employs a deep learning technique.

According a further advantageous embodiment the the striped pattern comprises a plurality of spaced-apart stripes on a background, wherein the stripes contrast with the background by any one or more of the respective colour, shade or intensity of the stripes and the background.

According a further advantageous embodiment the wherein the striped pattern comprises a plurality of stripes, wherein adjacent stripes contrast with each other by any one or more of their respective colour, shade or intensity.

According a further advantageous embodiment the method includes causing said backlighting illumination means to backlight said sample holder using structured light.

According a further advantageous embodiment the method includes including causing said backlighting illumination means to directly illuminate said sample holder.

According a further advantageous embodiment the method includes causing said indirect illumination means to illuminate said sample holder with diffused light.

The invention also sets out an apparatus for illuminating a colony-forming unit sample comprising:
means configured to receive a transparent or translucent sample holder containing colony-forming units;
at least one indirect illumination means configured to indirectly illuminate the transparent or translucent sample holder,
a backlighting illumination means configured to backlight the transparent or translucent sample holder,
and an image forming device positioned above said receiving means and configured to capture at least one image of the transparent or translucent sample holder.

According a an advantageous embodiment the image forming device is a camera

According a further advantageous embodiment the sample holder is a culture media plate.

According a further advantageous embodiment the apparatus includes means for processing the at least one image using a counting algorithm configured to analyse the least one image to count any colony-forming units rendered visible by the illumination means;
and reporting means for reporting the number of colony-forming units.

According a further advantageous embodiment the the counting algorithm employs a deep learning technique.

According a further advantageous embodiment the apparatus is a standalone colony counter.

According a further advantageous embodiment said at least one indirect illumination means is configured to illuminate said sample holder with diffused light.

According a further advantageous embodiment the, or each, indirect illumination means comprises at least one light source and at least one diffuser arranged to diffuse light from said at least one light source.

According a further advantageous embodiment said backlighting illumination means is configured to directly illuminate said sample holder.

According a further advantageous embodiment said backlighting illumination means is located below said receiving means.

According a further advantageous embodiment said backlighting illumination means is operable or configurable to provide patterned background lighting.

According a further advantageous embodiment said backlighting illumination means is operable or configurable to provide structured background lighting.

According a further advantageous embodiment said backlighting illumination means comprises a structured light projector.

While this disclosure has been set out in the context of an method and apparatus which employs indirect illumination in combination with backlighting, we note that a number of advantageous embodiments employ patterned lighting. The effects of patterned lighting noted herein are so marked that employing patterned lighting in an apparatus which does not use ambient or diffused lighting (e.g. using only backlighting or backlighting plus environmental lighting) would also benefit from the benefits of the patterned lighting embodiments set out herein.

Accordingly according to an alternative aspect, the following method is also described:

A method of illuminating a colony-forming unit sample, the method comprising:
backlighting a transparent or translucent sample holder containing colony-forming units with patterned lighting and capturing at least one image of the sample holder when backlit.

Such a method can also be amendment according to the advantageous embodiments set out herein, particularly by adding the additional features of the dependent claims.

The alternative aspect set out above would comprise the following corresponding apparatus:

An apparatus for illuminating a colony-forming unit sample comprising:

means configured to receive a transparent or translucent sample holder containing colony-forming units;
a backlighting illumination means configured to backlight a transparent or translucent sample holder wherein the backlighting illumination means is operable or configurable to provide patterned background lighting;
and an image forming device (60) positioned above said receiving means and configured to capture at least one image of the transparent or translucent sample holder.

Such an apparatus can also be amendment according to the advantageous embodiments set out herein, particularly by adding the additional features of the dependent claims.

Further advantageous aspects of the invention will become apparent from review of the following description of embodiments of the invention and with reference to the accompanying drawings.

Brief Description of the Drawings

Embodiments of the invention are now described by way of example and with reference to the accompanying drawings in which like numerals are used to denote like parts and in which:

Figures 1A and 1B show perspective views of an exemplary apparatus 10 for illuminating a colony-forming unit sample. ;

Figure 2A shows a sample holder containing CFUs illuminated by environmental lighting as known in the art.

Figure 2B is a line drawing depicting the sample holder of figure 2A and shows the CFUs as they would be recognised when illuminated by environmental lighting as is typical done in the art.

Figure 2C is a greyscale version of the image in figure 2A.

Figure 3A shows the same sample holder as that shown in figure 2A containing CFUs illuminated by according to the exemplary method set out herein.

Figure 3B is a line drawing depicting the sample holder of figure 3A and shows the CFUs as they would be recognise when illuminated according to the illumination of the exemplary method set out herein.

Figure 3C is a greyscale version of the image in figure 3A.

Figures 4A and 4B show an example petri dish illuminated by illumination means 40 with solid backgrounds of two or more different colours.

Figure 5 depicts a petri dish containing CFUs first illuminated using an orange background (figure 5A) and then a light blue background (figure 5B).;

Figures 6 A-D depict exemplary backgrounds which are illuminated using back lighting.

Figures 7 A-E depict exemplary backgrounds which are illuminated using back lighting from illuminations means 40.

Figure 7F depicts the same sample holder containing CFUs as in figures 2 and 3 illuminated using such a striped background.

Figure 7G is a line drawing of the sample holder of figure 7F depicting the CFUs identified by illuminating the sample holder according to the illumination method in conjunction with the striped background of figure 7F.

Figure 7H is a greyscale version of the image in figure 7F.

Figure 8A shows CFUs on an agar plate illuminated by environmental light only. Figure 8B shows the same agar plate, the only difference being that the sample is now illuminated according to the illumination method disclosed herein, whereby blue stripes have been specifically chosen.

Figures 9A and 9B show an example in which the sample holder is a petri dish illuminated by illuminations means 40 with a solid white background (figure 9A) and a background of patterned light formed by a striped pattern (figure 9B)

Figures 10A and 10B show another example in which the sample holder is a petri dish illuminated by illuminations means 40 with a solid white background (figure 10A) and a background of patterned light formed by a striped pattern (figure 10B.) To show the resultant CFUs more clearly figures 10A and 10B are also depicted as line drawings in figures 10C and 10D respectively. A further contrast is shown in figure 10E (white background) and figure 10F (striped).

Figure 11 depicts an instance in which illumination to create such a solid colour background (in this case yellow) was combined with darker lines and employed as the background in an exemplary apparatus according to the current invention to detect CFUs.

Figures 12A-E shows examples of how light is refracted differently resulting in a bend in the light when are sample are viewed.

Figures 13A and 13B show further examples of how having fine stripe lines will also enhance any small details that would otherwise be hidden

Figures 14 A, B and C show a particular sample imaged using different line and background colours.

Figure 15 depicts a variation with white lines on a black background.

In figure 16, the CFUs are illuminated using a coloured patterned background. Other possible patterns are shown in figures 17-19 which demonstrate how various background colours and patterns can be employed to enhance CFU detection.

Detailed Description of the Drawings

Typically, CFUs are counted manually. In a standard approach for counting a human operator examines sample holders in environmental lighting and performs a manual count. However, CFUs are difficult to identify. This disclosure sets out a method and apparatus method of illuminating a colony-forming unit sample. The improved illumination makes it easier to identify and therefore count CFUs.

With reference to the drawings, there is disclosed herein a preferred method of illuminating a colony-forming unit sample that embodies the invention and which employs a sample-appropriate method of illumination for accurate and reproducible detection of CFUs. By employing the illuminations techniques employed herein CFUs are illuminate such that CFUs can be counted which would not have been visible under environmental lighting. Consequently, counting accuracy is improved. While the microbial detection and identification applications described herein are focused on a method of illuminating a colony-forming unit sample, alternative applications known to persons skilled in the art may be chosen according to circumstances.

Also disclosed is a preferred embodiment of an apparatus comprising means for illuminating a colony-forming unit sample employing a sample-appropriate illumination method.

In a preferred embodiment the apparatus takes the form of a standalone colony counter.

Culture media & plate size

Samples are typically placed on plates such as petri dishes containing a suitable medium such as agar. Growth media with low selectivity i.e., capable of supporting a broad spectrum of microorganisms including bacteria, fungi, yeast, and moulds are typically for used for environmental monitoring and bioburden testing. It will be apparent to the skilled person that the method disclosed herein may have microbiological applications in cosmetics and food industries. When necessary to detect or search for a particular type of microorganism selective culture medium is used.

Media may also be modified and contain neutralizing agents to inactivate residual surface disinfectant present on the surface to be tested. Exemplary embodiments of this invention employ an agar as the culture medium; however, the method described herein is not limited to agar.

Alternative culture mediums known to persons skilled in the art and an alternative culture medium may be chosen according to circumstances. The recommended size of solid media is 90 mm in diameter (approximate internal area 64 cm^2) for settle plates and 55 mm (surface area 25 cm^2) for contact plates. The method described herein is also not limited to any particular plate size. Suitable sizes will be evident to the skilled person.

Automated CFU Counting Software and Associated Challenges

Manual CFU counting is a laborious time-consuming process that is subject to variability, accuracy, and precision and that is influenced by the colony morphology, colony density, and operator subjectivity. Accuracy can be defined as the ability of CFU counts to reflect the true value of the population and precision as the degree of reproducibility among the CFU counts.
Consequently, CFU counting may be automated. The illumination method and system set out herein is applicable not only to manual CFU counting but also to automated CFU counting. Such automated CFU counting is typically performed using a standalone CFU counting apparatus employing a counting algorithm. However, automated CFU counting software is particularly subject to the below challenges:

- o Differentiation of low count CFUs from background (important for sterility)
- o Accurate counting for high numbers of CFUs (100s)
- o Difficulty in accurate counting of CFUs growing on the edge of a sample holder
- o Differentiation between bacteria and fungi
- o Sensitive detection of pale coloured bacteria
- o Sensitive detection of early growth fungus

In addition to making CFUs more identifiable for manual counting, the method and apparatus set out herein seeks to address the issues above.

Accordingly, preferred embodiments of the invention provide sample-appropriate illumination method(s) and apparatus. Advantageously these may be applied to the problem of colony counting.

Preferably, the method disclosed herein is implemented using a colony counter apparatus, more preferably a standalone colony counter apparatus.

The apparatus may also comprise a computer readable medium comprising instructions that when implemented will control any one or more of the processes of image capture, a counting algorithm, report generation and network connection.

While the teaching described herein sets out an exemplary embodiment on a preferred apparatus, the skilled person would readily recognise that the advantages of the illumination method disclosed herein could be employed in known colony counters.

Preferred Sample-Appropriate Illumination Method and Apparatus for Illuminating a Colony-forming Unit Sample

Referring to figures 1A and 1B, the preferred method described herein is a sample-appropriate illumination method applied to the problem of Illuminating a Colony-forming Unit Sample. The method is carried out on an apparatus for illuminating a colony-forming unit sample In particular, the method is well suited to implantation in the form of a standalone apparatus.

Referring to figure 1A and 1B an apparatus 10 for illuminating a colony-forming unit sample according to an exemplary embodiment is described. The preferred illumination method is described in the context of such an apparatus. Alternative configurations of this exemplary apparatus will be readily conceived by the skilled person.

Figure 1A shows a perspective view of an exemplary apparatus 10 for illuminating a colony-forming unit sample. The apparatus comprises a stage or receptacle 50 configured to accept a transparent or translucent sample holder 52 52 containing colony-forming units.

One or more indirect illumination means 20 are provided. The illumination means 20 are configured to indirectly illuminate the transparent or translucent sample holder 52. The preferred apparatus further comprises a backlighting illumination means 40 configured to directly illuminate the transparent or translucent sample holder 52. An image forming device 60 is positioned above said stage or receptacle 50 and configured to capture images of the transparent or translucent sample holder 52, especially when illuminated (simultaneously) by the illumination means 20, 40. The image forming device 60 may also capture a portion of, or at least a portion of, the backlighting illumination means 40 below the sample holder 52 when capturing images of the sample holder 52.

In a method according to a exemplary embodiment, a colony-forming unit sample in a transparent or translucent sample holder 52 is illuminated by the one or more indirect illumination means 20 and the backlighting illumination means 40. The image forming device 60 captures at least one image of the transparent or translucent sample holder 52 and typically also the portion of the backlighting illumination means 40 below the sample holder 52.

The preferred illumination method according to the exemplary embodiment provides improved contrast, colour, and texture and so that CFUs can be more readily identified in the image, thereby enable improved colony counting, whether manual or automatic. This is demonstrated in the resultant images which are set out in the drawings. As the drawings depict the contrast, colour, and texture of the CFUs in the images, this are best depicted by colour photographs. Consequently

reference is made to colour photographs in the drawings. In addition, where helpful the images have also been reproduced in greyscale and/or monochrome line drawings.

As the sample holder 52 is transparent or translucent, when a sample is illuminated according to the method described herein, the images taken by the image forming device 60 will show the CFUs illuminated according to the indirect illumination means 20 and a background provided by backlighting illumination means 40.

Figure 2A shows a sample holder containing CFUs illuminated by environmental lighting as known in the art. In figure 2A, the sample holder 52 is not illuminated evenly. Moreover, the texture of the CFUS is not discernible. Furthermore, it is difficult to distinguish between the CFUs.

Figure 2B is a line drawing depicting the sample holder of figure 2A and shows the CFUs as they would be recognised when illuminated by environmental lighting as is typical done in the art.
Figure 3A shows the same sample holder as that shown in figure 2A containing CFUs illuminated by according to the exemplary method set out herein. In figure 3A, the sample holder is illuminated evenly. Furthermore, as the sample holder 52 is illuminated from below, the CFUs are much more visible than in figure 2A. The CFUs appear brightly and thus much more readily countable than in the corresponding image in figure 2A. The colour and shades of the individual CFUs can be seen.
Moreover, the illumination of the exemplary method set out herein of makes the texture of the CFUS is discernible. Since the CFUs are brighter and their individual colours and shades are evident, it is much easier to tell the CFUs apart. This is complemented by the visible texture whereby the three dimensional nature of the CFUs become more apparent. Therefore, not only can more CFUs be identified in figure 3A, they can readily be distinguished from each other. All of these factors facilitate the counting of the CFUs. The difference is especially evident in the section just above the centre of the sample holder. The different colours and textures in figure 3A permit more CFUs to be identified as clearly demonstrated in the corresponding line images 2B and 3B.

Figure 3B is a line drawing depicting the sample holder of figure 3A and shows the CFUs as they would be recognise when illuminated according to the illumination of the exemplary method set out herein. As can be seen in the figure, the CFUs are clearly distinguishable from each other and thus easier to count. Note that the three dimensional texture of the CFUs is absent in this depiction, yet the CFUs are nonetheless easier to count than in the corresponding image 2B.

Returning to figures 1A and 1B, the apparatus 10 preferably comprises a housing whereby side walls and the door 30 have been made transparent to permit depiction of the preferred internal components. However, like the side walls depicted in figure 1B the side walls should be non-translucent, or opaque, so that no external environmental light enters the apparatus during operation. For illustration purposes no door is depicted in figure 1B.

Looking to figure 1A, a receptacle 50 is depicted with a sample holder 52, in this case a petri dish, in situ for illustration purposes. The sample holder 52 may be configured to hold any type of plate. The sample holder 52 is positioned in the receptacle 50 above illumination means 40.

In an exemplary embodiment, the apparatus 10 comprises the backlighting illumination means 40 configured to provide illumination, preferably direct illumination, by backlighting the sample holder 52, and illumination means 20 configured to provide indirect lighting. The indirect illumination advantageously results in ambient lighting of the sample holder 52. Ambient lighting should be understood as lighting which comes from all directions in contrast with directional lighting, which is made up of a light means with parallel light rays that do not diminish with distance. Also, contrast with "positional lighting," in which the rays are not parallel, but diminish in intensity from the source. Advantageously, the indirect lighting comprises diffused light. To this end, the indirect illumination means 20 may comprise at least one light source and at least one light diffuser for diffusing the light provided by the light source. The, or each, diffuser may be of any conventional type, and may be a reflective diffuser or a transmissive diffuser as is convenient.

In the exemplary embodiment, the backlighting illumination means 40 comprises an electronically controlled display device on which images may be displayed. The illumination means 40 preferably comprises a structured light projector. However, the person skilled in the art would recognise that many known display devices may be used for this purpose. Typically, an LCD, OLED, TFT or similar display device is employed. The use of such a device provides direct illumination from below, i.e. backlighting, of the sample holder 52 carried by receptacle 50.

In the illustrated embodiment, a plurality of illumination means 20 a-f are configured to provide ambient illumination, i.e. light from all directions, the ambient light advantageously comprising diffused light. The plurality of illumination means 20 may be distributed around the housing in order to provide the ambient illumination. The ambient illumination is in contrast to the illumination provided by backlighting illumination means 40 which comes from below receptacle 50. In this exemplary embodiment, each ambient illumination means 20 a-f comprises a light source in the preferred form of one or more LEDs. The illumination means 20 a-f are preferably attached to the inner sides of the housing, or supported within the housing by any other convenient means. It will be evident to the skilled person that the number of lighting means 20, their position and intensity may be varied by the operators or automatically according to circumstances. It is thus possible to have no light at all emitted by illumination means 20 so that the sample holder 52 is illuminated only by the backlighting illumination means 40.

Each ambient lighting means 20 preferably comprises at least one diffuser which scatter the light. Each diffuser comprises a diffusing material which scatters the light thus ensuring that it illuminates surfaces evenly or substantially evenly. Each diffuser is preferably in the form of a translucent or semi-translucent cover of transmissive diffusing material which is placed over the individual illumination source of the illumination means 20 a-g. Alternatively, or in addition, one or more

reflective diffuser may be provided. The resultant lighting is diffused light or ambient light. Diffused light is a soft light with neither the intensity nor the glare of direct light. It is scattered and comes from all directions. It is softer and does not cast harsh shadows. The absence of such shadows has clear benefits for CFU detection as the colour, shade and texture all become much more well defined in the absence of shadows.

By contrast, backlighting illumination means 40 is below the receptacle 50 where the sample holder 52 containing the CFUs is to be placed. Preferably, illumination means 40 is configurable to provide illumination in a plurality of colours, shades and/or patterns. In this exemplary embodiment this is achieved by varying the image on the display. The illumination provided by illumination means 40 may vary in colour and/or shade. Its light may also be filtered by inlays or the like placed over the illumination means 40. These inlays may be patterned e.g. striped. The patterns described herein may be realised using either method e.g. by changing an image on a display or by changing the inlay to one with a different pattern and/or colour. In preferred embodiments, the illumination provided by the illumination means 40 comprises structured light. To this end, the illumination means may comprise a structured light projector. In preferred embodiments, the illumination means 40 is operable to provide varying illumination, i.e. a sequence of different patterns, shades and/or colours, wherein at least one respective image may be captured in respect of each different illumination. Alternatively, the illumination may be static. In any event, the illumination may be tailored to suit the contents of the sample holder 52.

The imaging device, or sensor, 60 is positioned above or substantially above the receptacle 50 for a sample holder 52 and is configured to capture images of any sample holder 52 inserted into the receptacle 50 and the CFUs contained therein.

As shown in figures 1A and 1B the lighting means 20 are preferably positioned on the walls of the housing as their function is to provide ambient light which is not directed at the imaging sensor 60. By contrast the imaging device 60 is located above illumination means 40 and the receptacle 50. Imaging device 60 is preferably a camera, but may comprise any imaging sensor known in the art.

In an alternative embodiment, the backlighting illumination means 40 is a backlighting illumination means such as an LCD illumination means. In this case the illumination means 40 is configured to permit an overlay be positioned over the illumination means such that light passes through the overlay. In a preferred embodiment one or more fixed pattern sheet is used for this purpose. As in the exemplary embodiment the colour of the light can be varied. Similarly, the overlay can be configured to provide variations in pattern, colour and/or shade of the illumination light from the illumination means.

Figure 1A further depicts a door 34 with hinges 32 which may be configured to open and close to permit manual insertion and removal of plates and the like.

Figure 1B further depicts an optional controller device 80 for controlling the apparatus, including changing the settings of the apparatus as required.

In operation a sample on a plate or similar is placed onto the receptacle 50 at the bottom of the apparatus below an imaging sensor 60. The imaging sensor 60 is preferably positioned centrally and supported on both sides by supports attached to either side of the inside of the housing walls.

Illumination intensity of Illumination means 20 a-g and backlighting illumination means 40 may be varied by an operator thus varying the intensity of both illuminations types on a sample on a sample holder. Such adjustments may be automated.

Note that, in operation, door 34 or any other opening will be closed such that no external environmental light illuminates the sample. Once the illumination is provided, imaging sensor 60 may take images of the sample. Either a single image may be taken or multiple images may be taken. In cases where multiple images are taken, the illumination settings may be varied i.e. in colour, shade and/or intensity for respective images. Furthermore, backlighting illumination means 40 may provide structured, or patterned, light either by providing a corresponding image on the electronic display means or structured projector, or by use of an overlay such as a fixed pattern sheet. Images are thus taken of CFUs on the sample. Images of a given sample may be taken with multiple different background illuminations and/or ambient light settings to facilitate CFU counting. The images of the samples may be displayed on an external display means to enable manual counting. Alternatively the apparatus may be configured to employ a colony counting algorithm to automatically count the colonies.

Preferred embodiments of the invention may employ an apparatus configured to carry out the counting automatically using a counting algorithm.

Illumination means 40 may comprise means of forming illumination patterns either by changing the image on the electronic display means, or provided by a structured light projector, or in an alternative embodiment by using a pattern on an overlay such as a fixed pattern sheet. In the latter case the overlay is positioned above illumination means 40 so that the light shines up and to an extent through the overlay. The overlay is transparent or translucent and comprise a pattern of lesser translucency. Examples of suitable patterns are shown in figures 6A-D and 7A-E.

As the sample holder is transparent or translucent, when a sample is illuminated using such a pattern images formed will show the CFUs illuminated according to the patterned background. An example of this is shown in figure 7F.

In preferred embodiments, the backlighting illumination pattern comprises stripes, preferably stripes with alternating colour and/or contrast. Such a pattern may be created by providing stripes on a display. Exemplary patterns using stripes are depicted for example in figures 6A-D and 7A-E.

Generally the stripes are parallel. Instead of such stripes non-straight lines may be employed; examples of such patterns are depicted for example in figures 17-20. Patterned illumination may also be realised using other patterns.

In preferred embodiments, the illumination method is applied specifically to colony counters. The colony counter of the exemplary embodiment is configured to count the CFUs identified by the illumination method. This may be achieved using any suitable conventional CFU (or other) counting algorithm(s), and by any suitable conventional counting means, e.g. a suitably programmed computer or other processor. Possible techniques are counting algorithms such as instance segmentation or object detection algorithms. The skilled person would be aware of many ways of doing this and, accordingly, this is not the focus of this disclosure.

Exemplary counting algorithms advantageously employ deep learning techniques, particularly deep learning algorithms for accurate and reproducible detection and counting of CFUs. A preferred embodiment of the invention employs sample-appropriate illumination for a deep learning CFU counting model method.

In an exemplary embodiment, an LED lighting configuration is employed which interacts with the topography and reflectivity of the CFUs a on plate, for example a TSA agar plate, to maximize the contrast, colour, and texture of the CFU features. The enhanced illumination method generates optimal imaging conditions for detecting and counting CFUs.

A preferred embodiment of the apparatus will also comprises a computer readable medium comprising instructions that when implemented will control the processes of image capture, a counting algorithm and report generation. Generally a network connection will be available to the apparatus.

The exemplary embodiment is specifically adapted for the sample-appropriate illumination for a CFU counting model application. The LED illumination configuration is designed to ensure that it interacts with the topography and reflectivity of the CFUs on the TSA agar plate to maximize the contrast, colour, and texture of the CFU features. The enhanced illumination solution generates optimal imaging conditions for detecting and counting CFUs.

In particular, the apparatus is arranged to ensure that back lighting can create contrast of the CFUs against the bright background of the couture medium (e.g. agar), while diffused lighting i.e. emulated ambient lighting enhances the topographic features of the CFUs. White light is composed of wavelengths of different colours, which enhances the colour vibrancy of the CFUs. The placement of the illumination configuration relative to the camera and agar plate creates the optimal sample-appropriate illumination configuration.

The placement of the illumination configuration relative to the camera and agar plate creates the optimal sample-appropriate illumination configuration. As can be seen from figure 1B, the imaging sensor e.g. that of a camera, is positioned substantially above the receptacle. Beneath the receptacle 50 is an illumination means. The illumination means 20 are positioned on the sides of such an apparatus. This setup permits illumination according to the pattern and/or colour of the light display. The imaging sensor is configured to take images of sample holders 52 inserted into the placeholder. As the pattern and or colour are illuminated by back lighting when the images are captured they will thus be visible in the background of the resultant images.

Illumination Configuration

The arrangement set out below preferably employs and an agar plate. A preferred agar plate is a TSA (Tryptic Soy Agar), a general-purpose medium, or SDA (Sabouraud Dextrose Agar). However, is evident to a skilled person that the particular plate and medium chosen will vary according to circumstances.

The arrangement of the illumination configuration relative to the camera and plate creates the preferred sample-appropriate illumination configuration. As can be seen from figures 1A and 1B, the imaging sensor 60 e.g. that of a camera, is positioned above or substantially above the receptacle 50 configured to received sample holders 52. Beneath the receptacle 50 is a backlighting illumination means 40 for providing the direct illumination from below. Illuminations means 20 are positioned on the sides of the apparatus of figures 1A and 1B. This configuration permits illumination according to the colour/shade and/or pattern of the illumination source for providing the patterned illumination. The imaging sensor 60 is configured to take images of sample holders 52 such as plates inserted into the receptacle 50. The pattern and/or colour of the illumination source for providing the patterned illumination will thus be visible in the background of the resultant images. Note the distinction between colours can also me made through different shades of colours.

Illumination Configuration For Optimal Colour And Texture Of CFU Features

The exemplary Illumination method utilizes back lighting from illumination means 40 to create contrast of the CFUs against the bright background of the culture medium (e.g. agar), while ambient lighting provided by illumination means 20 enhances the topographic features of the CFUs. White light is composed of wavelengths of different colours, which enhances the colour vibrancy of the CFUs. As stated above, the placement of the illumination configuration relative to the camera and agar plate creates the optimal sample-appropriate illumination configuration. As can be seen from figures 1A and 1B, the imaging sensor 60, is positioned substantially above the receptacle 50. Beneath the receptacle 50 is an illumination means 40. Illumination means 20 are positioned on the sides of such an apparatus. This setup permits illumination according to the pattern and/or colour of the light display. The imaging sensor is configured to take images of sample holders 52 inserted into

the receptacle 50. The pattern and/or colour/shade will thus be visible in the background of the resultant images.

Backlighting Illumination means 40 is positioned such that a sample holder 52 inserted into receptacle 50 may be illuminated from below such that the CFUs are viewable as shown in figure 3A. Contrast figure 2A which shows how CFUs on an agar plate illuminated without the illumination method disclosed herein with figure 3A shows the same agar whereby the CFUs on the agar plate are illuminated with illumination method of the exemplary embodiment. Looking to figure 3A, the back lighting from the illumination means 40 results in a contrast of the CFUs against the bright background of the culture medium (e.g. agar), while the ambient lighting of illumination means 20 has enhanced the topographic features of the CFUs. By employing the illumination configuration employing direct lighting from below using illumination means 40 and ambient lighting from illumination means 20, the colour vibrancy of the CFUs in figure 15 is thus enhanced, enabling a more accurate count.

Solid Backgrounds Of Different Colours

Figures 4A and 4B show an example petri dish illuminated by backlighting illumination means 40 with solid backgrounds of two or more different colours in this case purple (fig 4A) and yellow (fig 4B) by way of example. In this case by changing the background colour whilst maintaining a solid background in each case different aspects of the CFUs and indeed different colonies become visible. Thus by taking images of the petri dish (or other sample) with two or more differently coloured backgrounds in sequence, and preferably filtering out any duplicates, a more accurate count of the CFUs can be made.

It should be noted, however, that is some cases, depending on the colour of the sample, the background colour employed by backlighting illumination means 40 can either amplify the CFU or can help hide it, meaning that in this case the results are mixed based on each case and are not as generic as illumination using a white background. This can be seen in the example shown in figures 5A and 5B in which a petri dish containing CFUs is first illuminated using an orange background (figure 5A) and then a light blue background (figure 5B). It will, however, be evident to the skilled person that the background colour may be optimally selected according to the particular sample taken in a given case.

Patterned And Or Coloured Illumination For Optimal Detection Of Early Growth Fungus

Early growth fungus and certain colonies that are translucent in morphology, can be challenging to detect against a bright background. The use of a striped backlighting creates an opposing colour contrast that enhances detection of the CFUs against the patterned light background.

In a preferred embodiment, striped patterned backlighting is employed e.g. in the form of parallel stripes. While horizontal stripes are preferred although the skilled person will readily see that e.g.

vertical stripes may be employed instead. The stripes should be parallel or substantially parallel to each other.

The following figures demonstrate how the backlighting contrasts between the brightness of the light and the relatively darker lines employed as stripes. As was the case above, white light is preferred, but it should be noted that any brighter colour will have similar effect. The important aspect is the contrast between the relative brightness of the light e.g. white light appearing white and the relatively darker colour of the stripes. In the examples below black and blue stripes are shown as being exemplary in this respect. The results shown with respect to black and blue below can be deemed indicative of a darker shaded strip win comparison to the brightness e.g. whiteness of the light.

Each of the patterns depicted herein may be realised by using a backlighting illuminations means 40 which is a display on which the images are directly displayed or with a backlighting illumination means 40 which is a lighting means onto which an inlay or the like is placed. In the latter case the inlay or the like would be created with one of the patterns thereon. In the former case what is termed the “background” is the light part of the image (white in figures 6A-D below).

Figures 6 A-D depict exemplary backgrounds which are illuminated using back lighting. In these figures black lines contrast with a white background.

As can be seen in Figure 6A, the black lines are thicker than those formed by the remaining visible background. Note also how the black colour may fade gradually into the white background.

In figure 6B the black lines are thinner than those in figure 6A and there are more of them.

In figure 6C a line pattern has been employed whereby black lines of varying widths are used in a repeating pattern.

Figure 6D depicts an alternative configuration in black lines of varying widths are used in a repeating pattern.

Figures 7 A-E depict exemplary backgrounds which are illuminated using back lighting from illuminations means 40. In these figures blue lines contrast with a white background.

As can be seen in figure 7A, the lines are arranged in a manner similar to that shown in figure 301A in that the blue lines are thicker than the lines formed by the remaining visible background. Note also how the stripes are arranged so that the blue colour may fade gradually into the lines formed by the white background.

In figure 7B the blue lines are thinner than those in figure 7A and there are more of them.

In figure 7C, again the blue lines are thinner than those in figure 7A; however they are thicker than the lines formed by the remaining visible background.

In figure 7D, a line pattern has been employed whereby thicker and thinner blue lines are used in combination. In this case the lines formed remaining visible background are thicker than even the thickest of the blue lines.

In figure 7E, an example is provide in which the blue lines are sharply defined such as they do not fade gradually into the white background i.e. they are straight and parallel to each other. As will be obvious to the skilled person, other shades of blue and other colours such as black may also be used to create images of the pattern depicted in figure 7E.

Figure 7F depicts a sample holder containing CFUs illuminated using such a striped background.

Figure 7G is a line drawing of the sample holder of figure 7F depicting the CFUs identified by illuminating the sample holder according to the illumination method in conjunction with the striped background of figure 7F.

Figure 7H is a greyscale version of the image in figure 7F.

Patterned Light Background

The following examples demonstrate how the patterns shown in figures 6 and 7 are employed in the illumination method as set out herein. In particular, the following examples highlight the advantages of a striped background whereby the stripes are parallel or substantially parallel. The embodiment set out below employs a striped pattern. It will be evident to a person skilled in the art that any of the patterns shown in figures 6 and 7 and patterns with similar characteristics will yield similar effects.

The technical effect of the striped pattern is even more clearly in evidence when a close up of a portion of a sample illuminated according to the illumination method disclosed herein compared to the same sample illuminated by environmental light only. Such a comparison is made in figures 8A and 8B . Figure 8A shows CFUs on an agar plate illuminated by environmental light only. Figure 8B shows the same agar plate, the only difference being that the sample is now illuminated according to the illumination method disclosed herein, whereby blue stripes have been specifically chosen.

Furthermore, a CFU which was not at all visible when the sample was illuminated under environmental lighting is clearly visible and countable in figure 8B. The CFU can be seen on the lowest stripe of figure 8B, to the left of the bottom of the largest CFU depicted. Above and to the left of that CFU is a narrow CFU extending up and to the left. While that CFU is clearly visible in figure 8B, the CFU below it is not. It is only made visible by the blue line which provides a contrast to the white light. This configuration is particularly improves early fungus detection.

Figures 8A and 8B are also depicted a line drawings in figures 8C and 8D respectively. As could be seen in figures 8A and 8B, The additional CFU is clearly visible in figure 8D but not at all in figure 8C.

Other features that are virtually impossible to see on just the white background are clearly visible under the pattern. It is clear how the light has been bent yielding around the edges of the CFUs improved definition of the CFUs. This is the result of the CFUs eating the agar which acts as a lens. Since the agar acts as a lens and some of it is eaten by the CFUs, the light is refracted differently resulting in a bend in the light when the sample is viewed. The resultant bend in the light is particularly apparent when the sample is illuminated with a patterned backlighting. This bending of the light, which is more readily viewable because of the striped background, is a further reason why using a patterned background leads to good identification of CFUs. The bend is particularly pronounced in the line just below the centre of figure 7F. Again the lines bend the light so that the CFUs are more clearly defined. In this case, however, the specific choice of blue lines makes the CFUs particularly visible.

Figures 9A and 9B show an example in which the sample holder is a petri dish illuminated by illuminations means 40 with a solid white background (figure 9A) and a background of patterned light formed by a striped pattern (figure 9B) to create an opposing colour or shade contrast. Comparing the two images clearly demonstrates how colonies are missed when set against a plain bright background but are visible and thus countable when a background of white light with black stripes is employed. It can be seen that more details visible in the image of figure 9B in comparison with the image of figure 9A. In particular, the white mould becomes easier to see by having the striped line pattern in the background. This is particularly evident in the large white CFU which is clearly visible on the right of figure 9B, but not visible at all on the right of figure 9A.

Similar effects can be seen when a contrast is made between figures 10A and 10B. To show the resultant CFUs more clearly figures 10A and 10B are also depicted as line drawings in figures 10C and 10D respectively. A similar result is evident when figure 10E (white background) is compared with figure 10F (striped).

As can be seen in figures 9 and 10 the illumination method set out herein is particularly effective at revealing pale coloured CFUs which would be difficult or impossible to identify in environmental light. The illumination methods set out herein are thus particularly suitable for the detection of pale coloured bacteria.

The effects of patterned lighting noted herein are so marked that employing patterned lighting in an apparatus which does not use ambient or diffused lighting (e.g. using only backlighting or backlighting plus environmental lighting) would also benefit from the benefits of the patterned lighting embodiments set out herein. In an alternative aspect, the method and apparatus should be understood in the context of the sample holder 52 being illuminated only by backlighting from

illumination means 40 or by such backlighting in conjunction with some environmental lighting. In this aspect, the backlighting is patterned lighting.

Solid Colour Background Combined With Lines

Figure 11 demonstrates an instance in which illumination to create such a solid colour background (in this case yellow) was combined with darker lines and employed as the background in an exemplary apparatus according to the current invention to detect CFUs. While a bend in the light is also evident when such coloured backgrounds are employed, the light is not refracted as well as when a solid white background is employed. This is due to the limited spectrum of wavelengths. When using light combined with a striped background, a white background is preferred for this reason.

Different Line Pattern Gaps

As noted above, there are features that are essentially impossible to see on just the white background whereas they become readily visible when illuminated using a pattern. This is the result of the lines bending the light. Figures 12 A-E demonstrated clearly how the light is bent and how this makes the CFUs visible and defined. Further, in figures 12 A-E, the gaps between the lines get progressively bigger.

The contrast between figure 13A (plain white background) and figure 13B provides further examples of how having fine stripe lines will also enhance any small details that would otherwise be hidden. Generally speaking, the thinner the lines (and therefore the more of them) the better. However, this comes with a trade-off that having more lines means having less light passing through from the backlighting illumination means 40 to the receptacle 50 containing the sample holder. The pattern spacing may therefore be adjusted according to the needs of the application. This may cause the pattern spacing to vary somewhat depending on the particular setup capabilities.

In typical embodiments, an optimal configuration is 50% line / 50% background light with a line width of 0.5mm. The background light is preferable realised by illuminating with illumination means 40 using white light only. Of course, the skilled person will adapt such an optimal configuration according the results shown for particular colours and shades set out herein. In addition, the skilled person with the normal means and capacity for routine work and experimentation would adapt such an optimal configuration according to circumstances.

It will be apparent that the patterns shown herein are used advantageously in the context of both manual colony counting and an automatic colony counter configured to implement a counting algorithm. Particularly in implementations using such a counting algorithm, the illumination can be changed dynamically and imaged. CFUs may be detected with a plurality of different illumination patterns, and combined to get the total number of CFUs, preferably with the counter being

configured to reject any detected duplicates. The advantages of multiple colours, shades and/or line spacing can be combined to arrive at a more accurate final count. Of course such a comparative approach is also useful when counting is performed manually.

Line Colour

As noted above, more details are visible in the image taken using the patterned illumination as opposed to the image taken using the illumination with a solid white background. As already explained above, this is the result of the culture medium, here agar, acting as a lens and the light being refracted differently resulting in a bend in the light when the sample is viewed.

The examples described above all used black lines for this purpose. Black lines give the most contrast and are thus generally the easiest to work with. However, other colours may be employed for the lines. In an alternative preferred embodiment, which also provides good results, blue lines are employed. The examples described above all used horizontal lines for this purpose, the skilled person will readily see that e.g. vertical stripes may be employed instead.

It should be noted that while black or blue lines are set out as preferred examples, some patterns may be better for certain types of sample holder. Another useful approach is to apply a series or sequence of patterns, especially different patterns, to highlight different types of CFUs.
Figures 14 A, B and C show a particular sample imaged using different line and background colours.
Figure 14B depicts the sample illuminated using white light and brown lines.
Figure 14B depicts the sample illuminated using pale green light and brown lines.
Figure 14C depicts the sample illuminated using pale green light and dark green lines.
In each case different aspects of the CFUs become visible. Such an approach is preferably applied to a variation of the method whereby multiple images are taken and compared to arrive at a final colony count.

Another variation is to employ white lines on a black background. This creates an X-Ray-like effect. However, it may provide variable results in terms of smaller darker CFUs. An example is shown in figure 15.

Image Patterns Instead Of Line Patterns

As noted above, early growth fungus and certain colonies that are translucent in morphology, can be challenging to detect against a bright background. The use of a striped patterned backlighting creates an opposing colour or shade contrast that enhances detection of the CFUs against the patterned light background. This section sets out exemplary backgrounds which have been shown to improved CFU detection and thus improved automatic colony counting.

One embodiment employs images that have colour patterns in association with backlighting illumination means 40. An example is shown in figure 16 in which the CFUs are illuminated using a coloured patterned background.

Other possible patterns are shown in figures 17-19 which demonstrate how various background colours and patterns can be employed to enhance CFU detection.

The background may be changed to any of the backgrounds described herein. Backlighting illumination means 40 are thereby configured such that they may be changed dynamically. The sample holder 52 exposed to each instance of lighting and background may be imaged by the imaging device. CFUs can be detected with each illumination pattern and or light colour and combined to get the total number of CFUs. In such case the software is configured to reject duplicates.

The skilled person would recognise that the backgrounds, colours, shades and patterns shown herein should not simply be viewed in isolation but also in the context of an apparatus which may employ a counting algorithm. In such an implementation, The illumination can be changed dynamically and imaged. CFUs can be detected with each illumination combination and combined to get the total number of CFUs with the software rejecting duplicates. The advantages of multiple patterns can thereby be combined to arrive at a more accurate final count.

The skilled person will recognise that by the method as set out above improves the contrast, colour, and texture and so that CFUs can be more readily identified in the image. In particular it should be noted that the skilled person will realise that the backgrounds and patterns can be varied depending on a particular the particular CFUs expected in the sample. For example the background colour can be changed to reflect the colour of a specific bacteria or fungus enabling better differentiation of low count CFUs. Differentiation of low count CFUs is important for sterility. This is particularly relevant in the case of pale coloured bacteria.

By facilitating the differentiation of CFUs facilitates accurate counting for high numbers of CFUs particularly when hundreds of CFUs are present.

The improved contrast, colour, and texture yielded by the illumination method facilitates the differentiation between bacteria and fungi.

The invention is not limited to the embodiment(s) described herein but can be amended or modified without departing from the scope of the present invention as defined by the appended claims.

CLAIMS:

1. A method of illuminating a colony-forming unit sample, the method comprising:
illuminating a transparent or translucent sample holder (52) containing colony-forming units using at least one indirect illumination means (20) and backlighting illumination means (40);
capturing at least one image of the transparent or translucent sample holder (52) when illuminated by said at least one indirect illumination means and said backlighting illumination means.
2. The method of claim 1, including causing the backlighting illumination means (40) to backlight said sample holder (52) using uniform lighting in a single colour.
3. The method of claim 1, including causing the backlighting illumination means (40) to backlight said sample holder (52) using patterned lighting.
4. The method of claim 3 wherein the patterned lighting comprises a striped pattern.
5. The method of claim 4, wherein the striped pattern comprises parallel stripes.
6. The method of claim 4 or 5 wherein the striped pattern comprises a plurality of spaced apart stripes on a background, the stripes being darker than the background.
7. The method of any of claims 4-6 wherein the striped pattern comprises a plurality of stripes on a white background.
8. The method of any of claims 4-7, wherein the striped pattern comprises black stripes.
9. The method of any of claims 4-7, wherein the striped pattern comprises blue stripes.
10. The method of any preceding claim, including capturing a plurality of images in sequence, and causing said backlighting illumination means (40) to provide respective different backlighting illumination for each image.
11. The method of claim 10 wherein the plurality of images in sequence comprises patterned lighting and/or uniform lighting in a single colour or a combinations of both.
12. The method of any preceding claim, wherein the, or each, image is processed and wherein the processing comprises employing a counting algorithm which analyses the, or each, image to count any colony-forming units rendered visible by the illumination; and reporting or recording the number of colony-forming units.
13. The method of any of claim 12, wherein the counting algorithm employs a deep learning technique.
14. The method of any one of claims 3-13, wherein the striped pattern comprises a plurality of spaced-apart stripes on a background, wherein the stripes contrast with the background by any one or more of the respective colour, shade or intensity of the stripes and the background.
15. The method of claim 4, wherein the striped pattern comprises a plurality of stripes, wherein adjacent stripes contrast with each other by any one or more of their respective colour, shade or intensity.
16. The method of any preceding claim, including causing said backlighting illumination means (40) to backlight said sample holder (52) using structured light.

17. The method of any preceding claim, including causing said backlighting illumination means (40) to directly illuminate said sample holder (52).
18. The method of any preceding claim, including causing said indirect illumination means (40) to illuminate said sample holder (52) with diffused light.
19. An apparatus (10) for illuminating a colony-forming unit sample comprising:
means (50) configured to receive a transparent or translucent sample holder (52) containing colony-forming units;
at least one indirect illumination means (20) configured to indirectly illuminate the transparent or translucent sample holder (52),
a backlighting illumination means (40) configured to backlight the transparent or translucent sample holder (52),
and an image forming device (60) positioned above said receiving means (50) and configured to capture at least one image of the transparent or translucent sample holder (52).
20. The apparatus of claim 19, wherein the image forming device (60) is a camera.
21. The apparatus of claim 19 or 20, wherein the sample holder (52) is a culture media plate.
22. The apparatus of any of claims 19-21 further comprising
means for processing the at least one image using a counting algorithm configured to analyse the least one image to count any colony-forming units rendered visible by the illumination means (20, 40);
and reporting means for reporting the number of colony-forming units.
23. The apparatus of claim 22, wherein the counting algorithm employs a deep learning technique.
24. The apparatus of any one of claims 19 to 23, wherein the apparatus is a standalone colony counter.
25. The apparatus of any one of claims 19 to 24, wherein said at least one indirect illumination means (20) is configured to illuminate said sample holder (52) with diffused light.
26. The apparatus of claim 25, wherein the, or each, indirect illumination means (20) comprises at least one light source and at least one diffuser arranged to diffuse light from said at least one light source.
27. The apparatus of any one of claims 19 to 26, wherein said backlighting illumination means (40) is configured to directly illuminate said sample holder (52).
28. The apparatus of any one of claims 19 to 27, wherein said backlighting illumination means (40) is located below said receiving means.
29. The apparatus of any one of claims 19 to 28, wherein said backlighting illumination means (40) is operable or configurable to provide patterned background lighting.
30. The apparatus of any one of claims 19 to 29, wherein said backlighting illumination means (40) is operable or configurable to provide structured background lighting.
31. The apparatus of claim 30, wherein said backlighting illumination means (40) comprises a structured light projector.

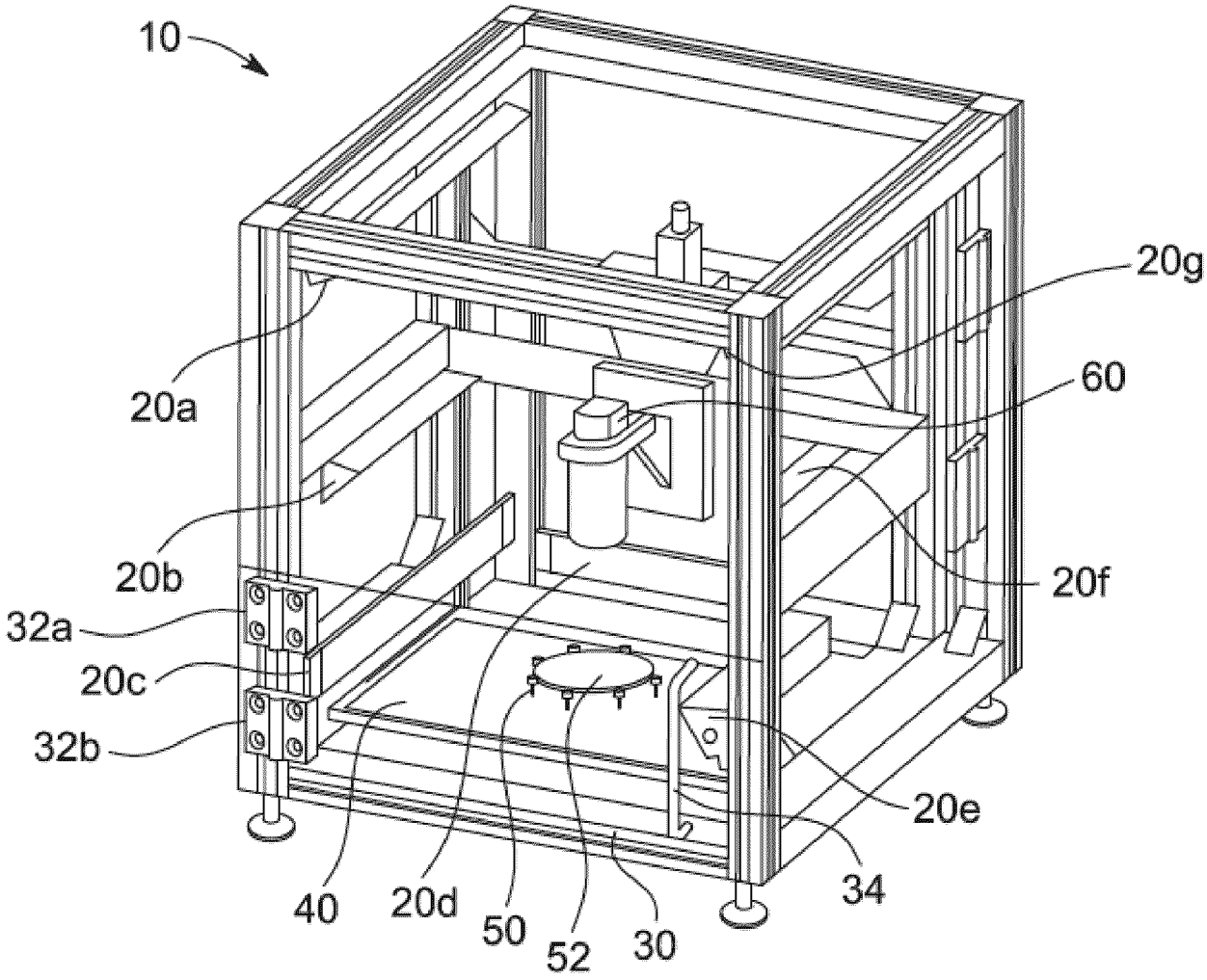

FIG. 1A

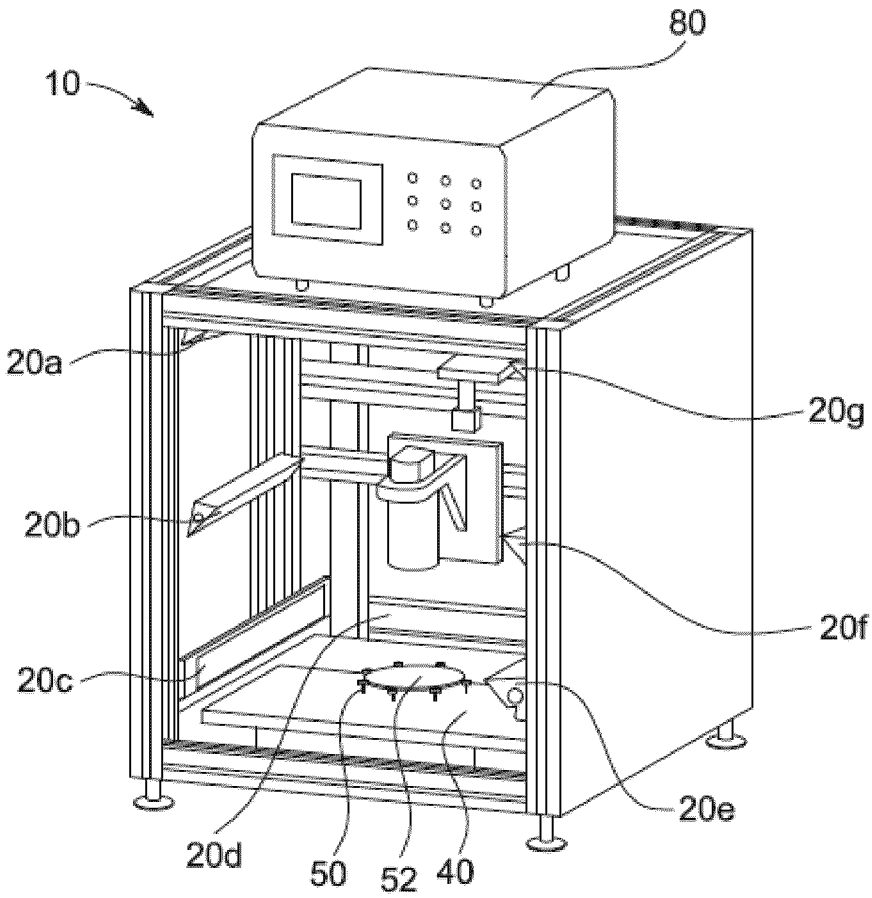

FIG. 1B

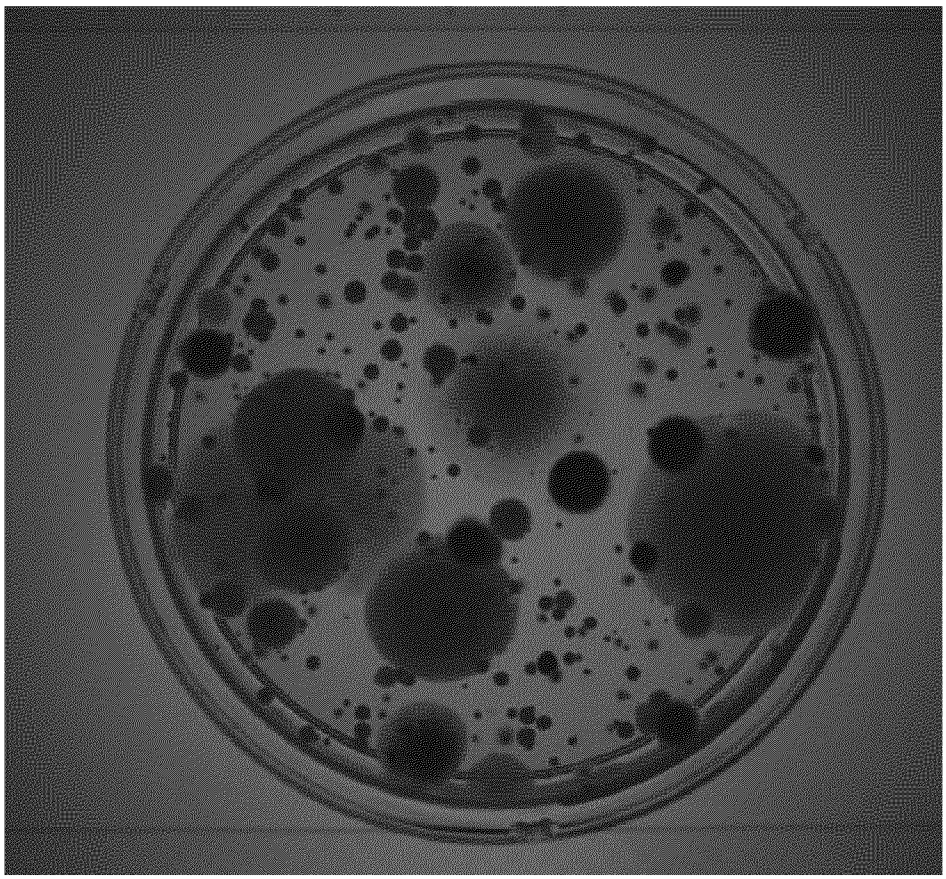

FIG. 2A

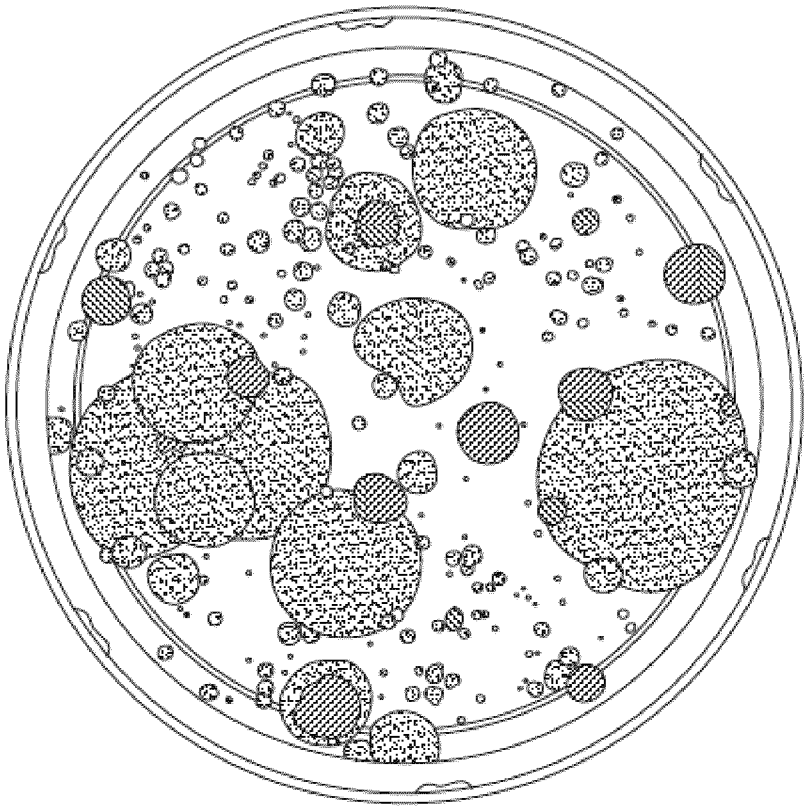

FIG. 2B

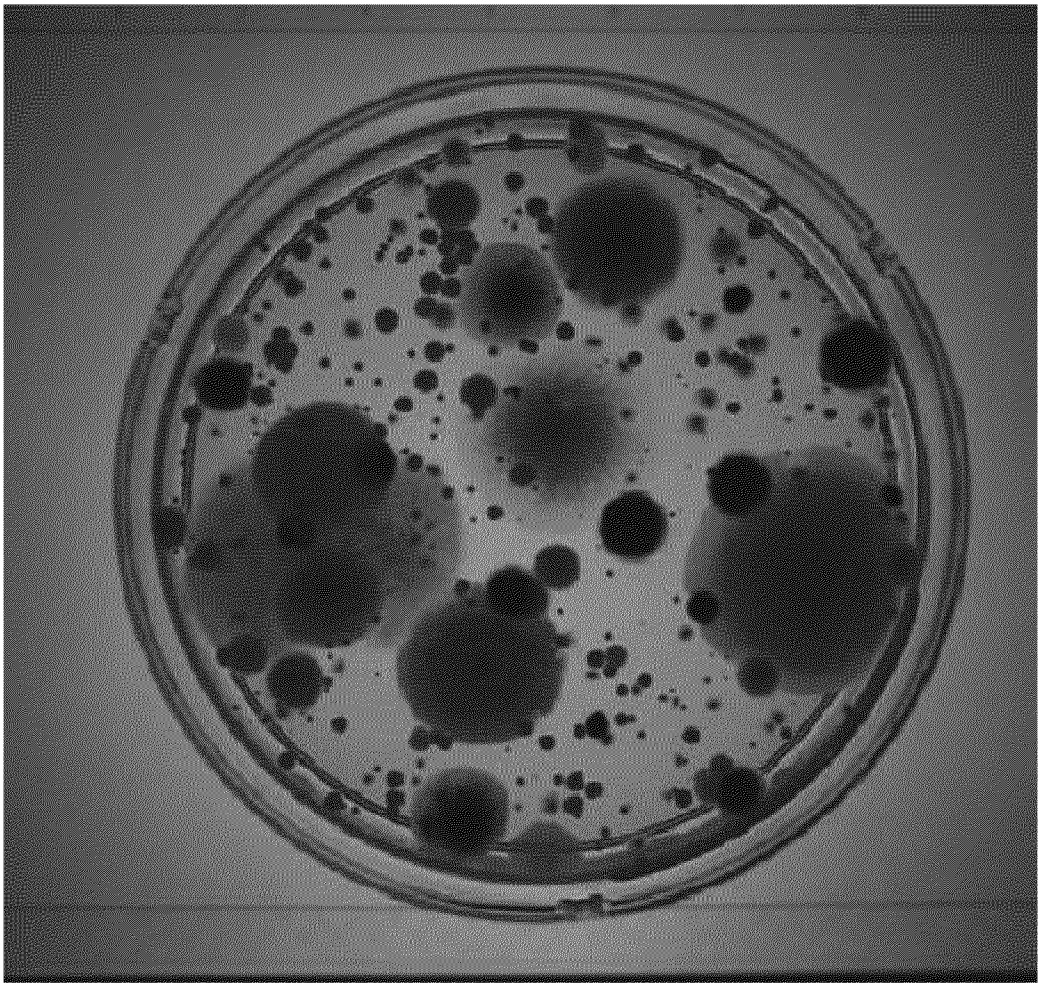

FIG. 2C

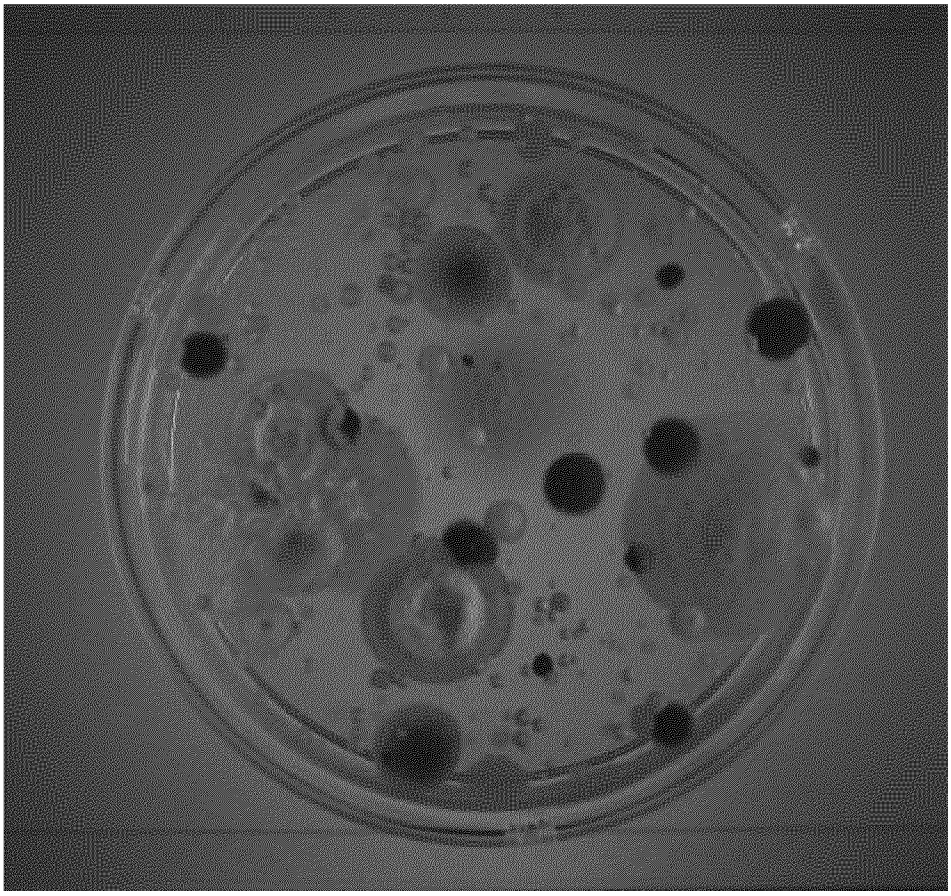

FIG. 3A

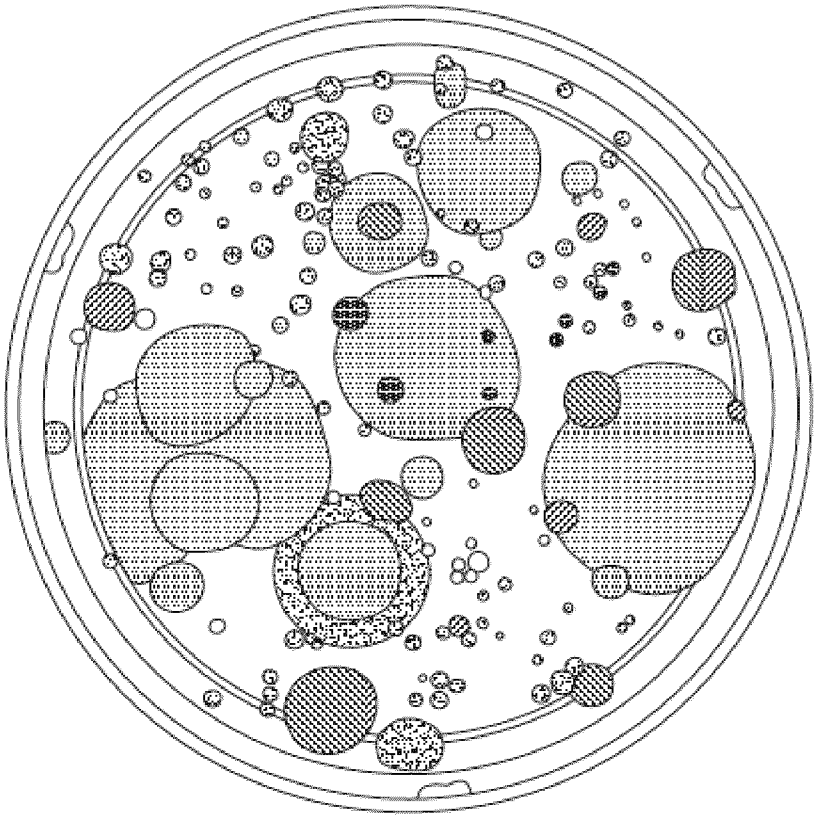

FIG. 3B

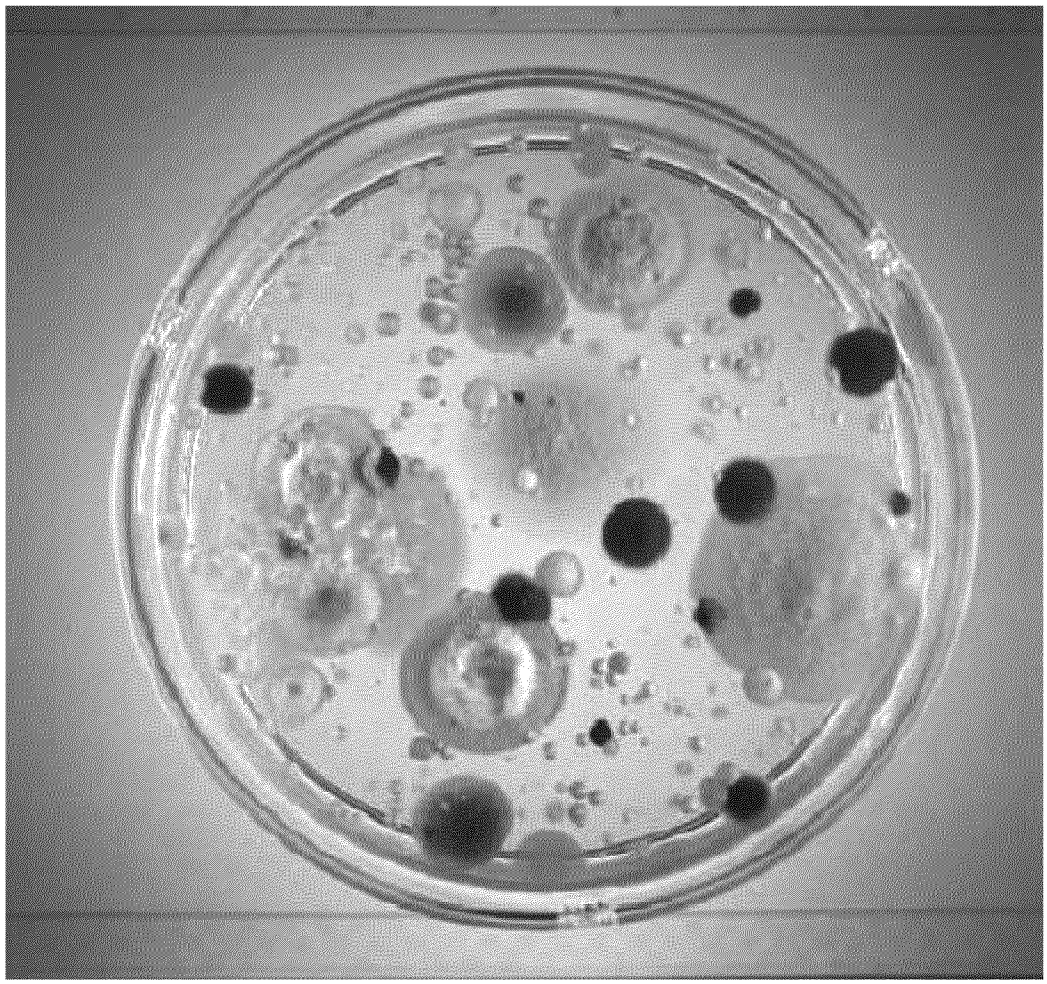

FIG. 3C

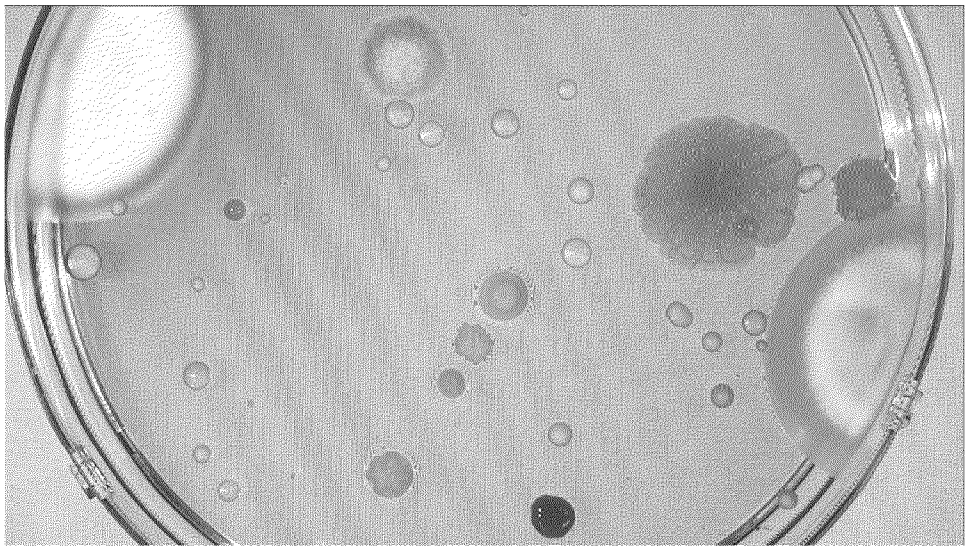

FIG. 4A

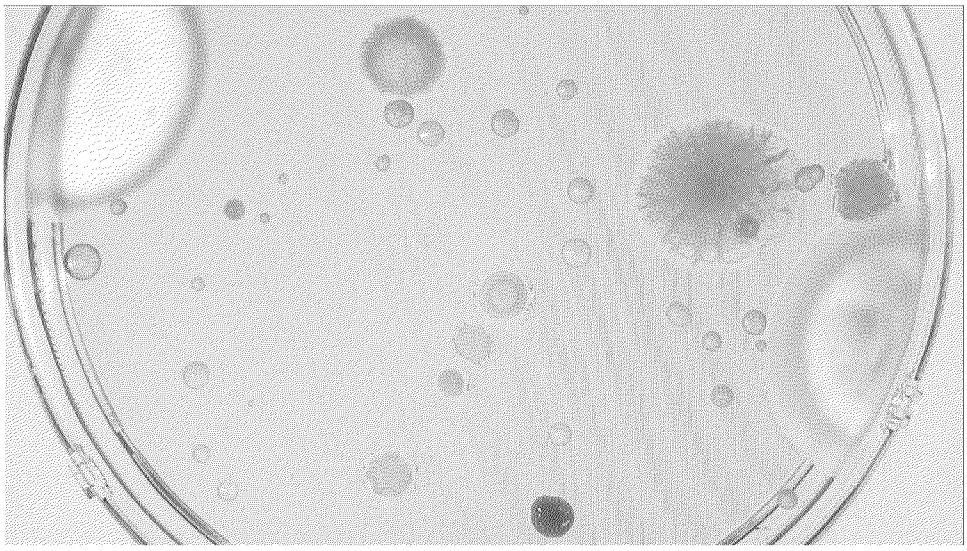

FIG. 4B

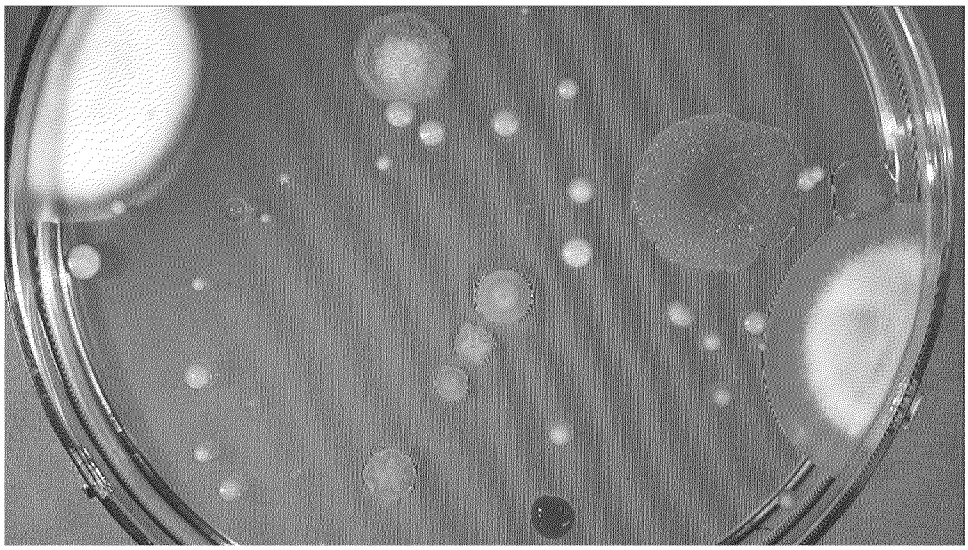

FIG. 5A

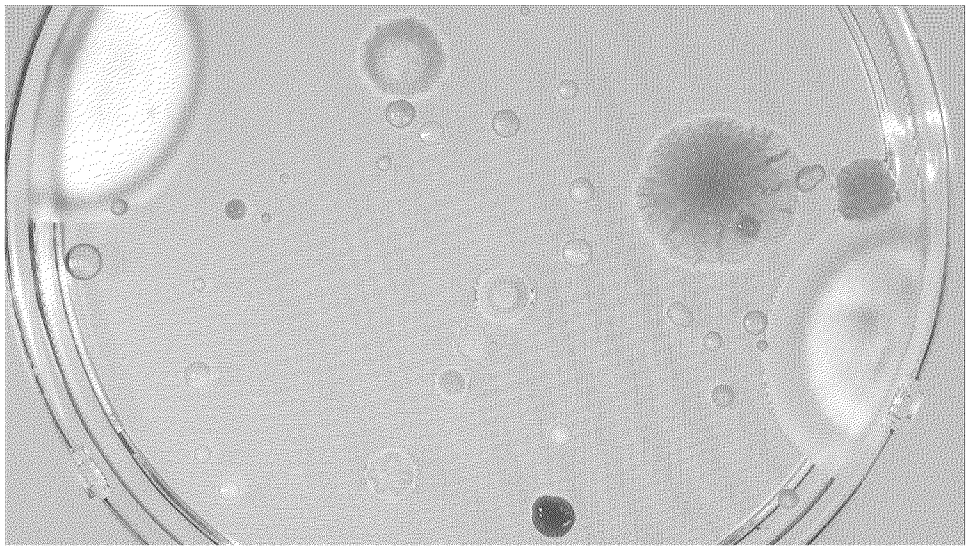

FIG. 5B

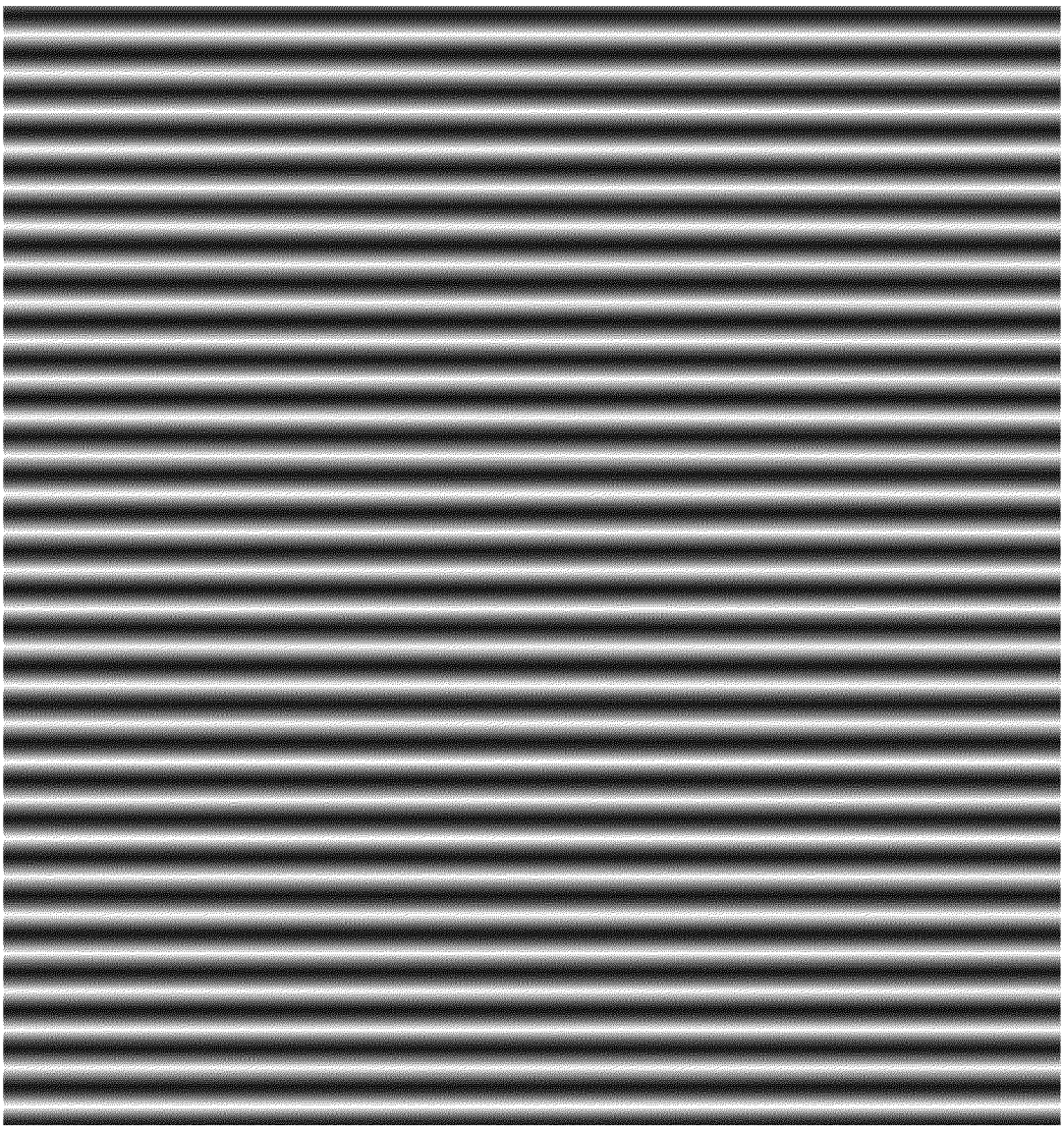

FIG. 6A

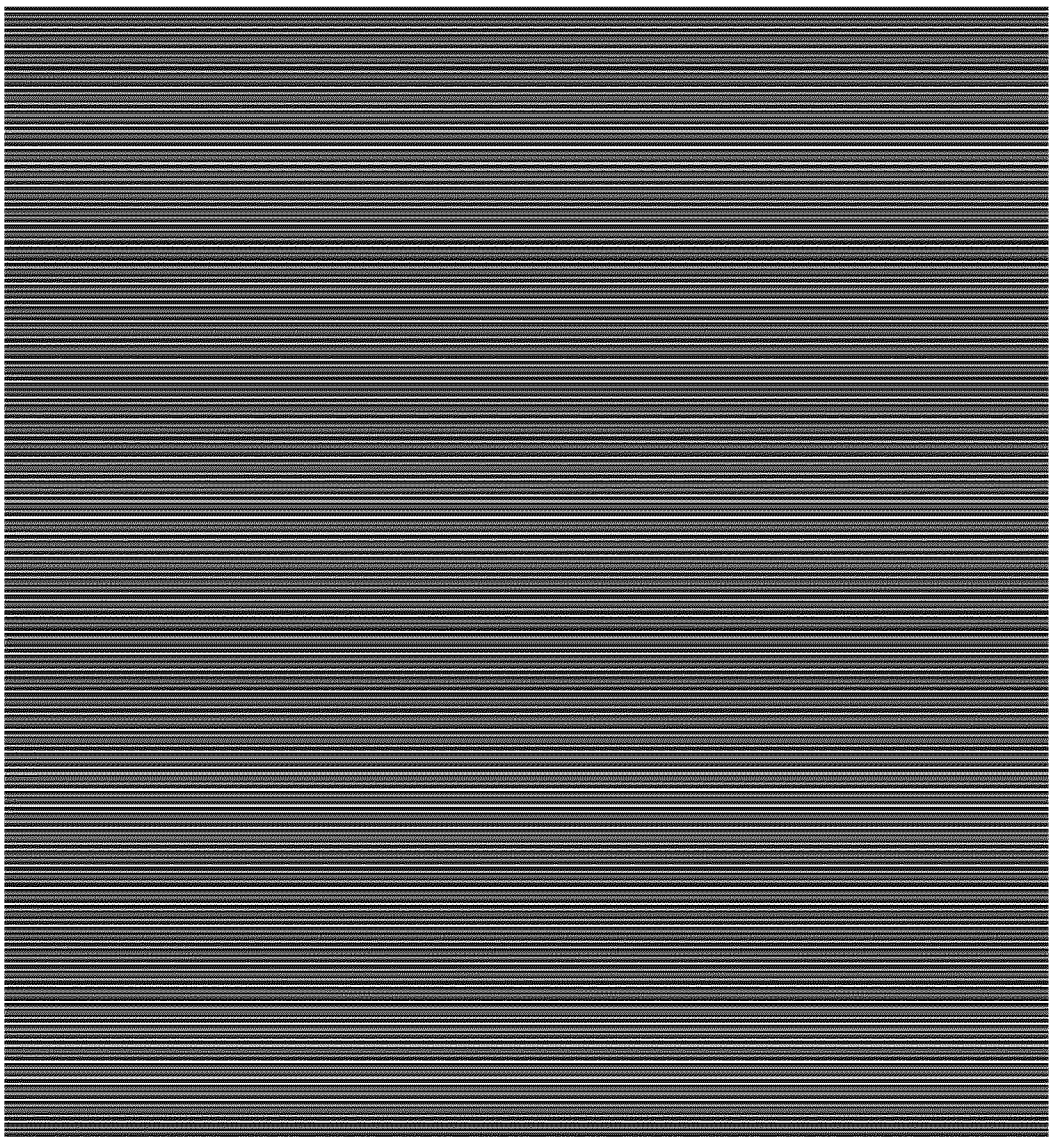

FIG. 6B

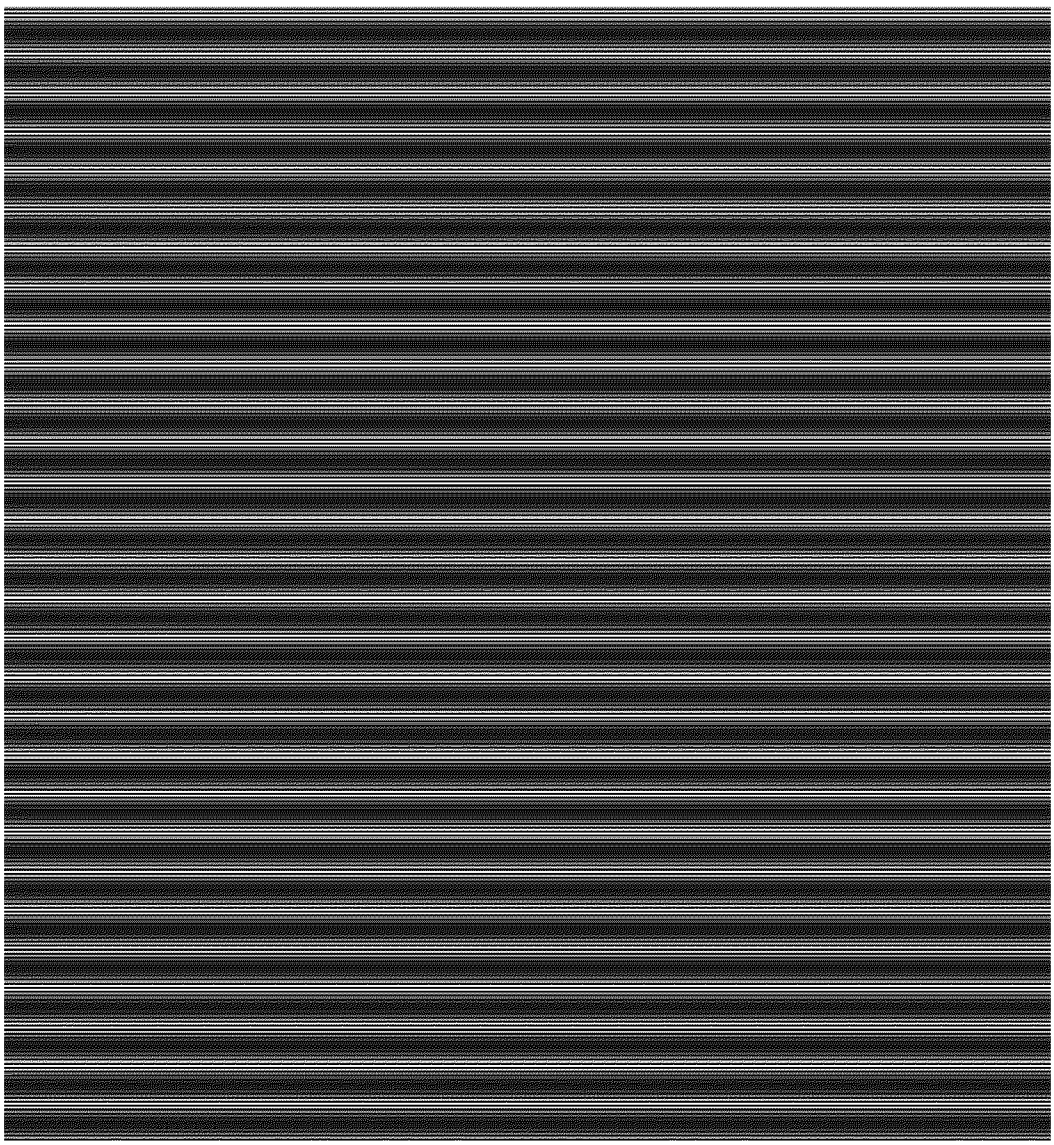

FIG. 6C

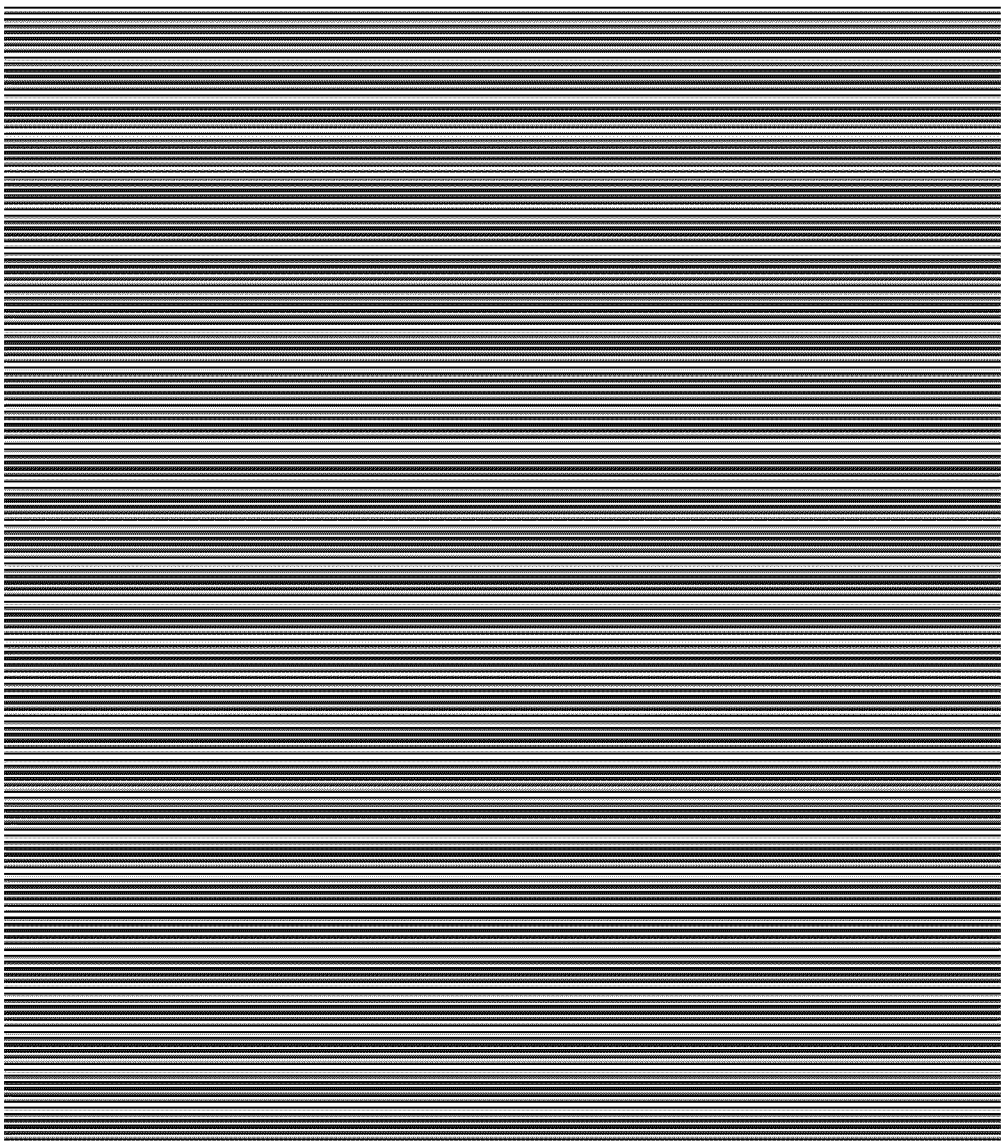

FIG. 6D

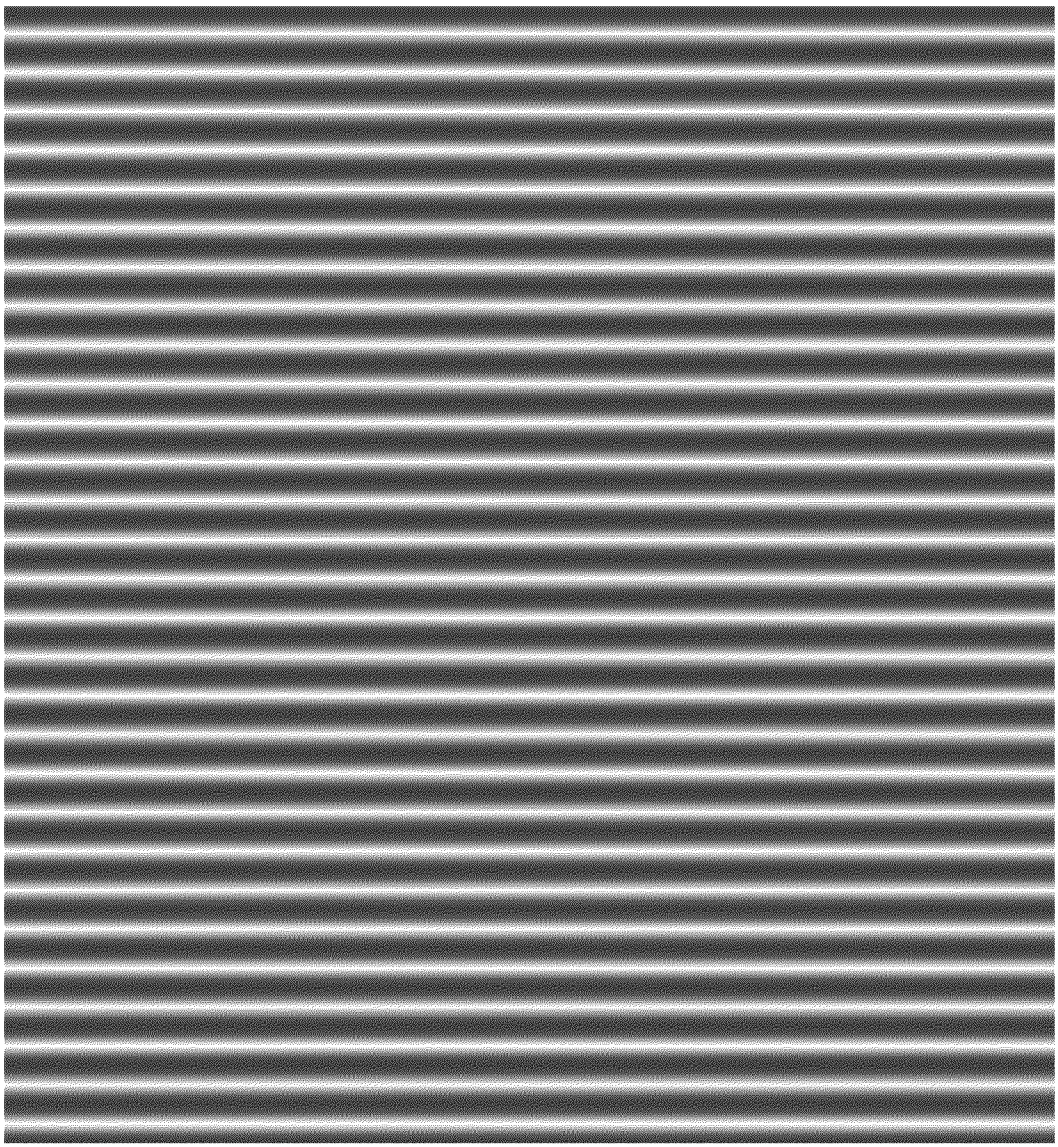

FIG. 7A

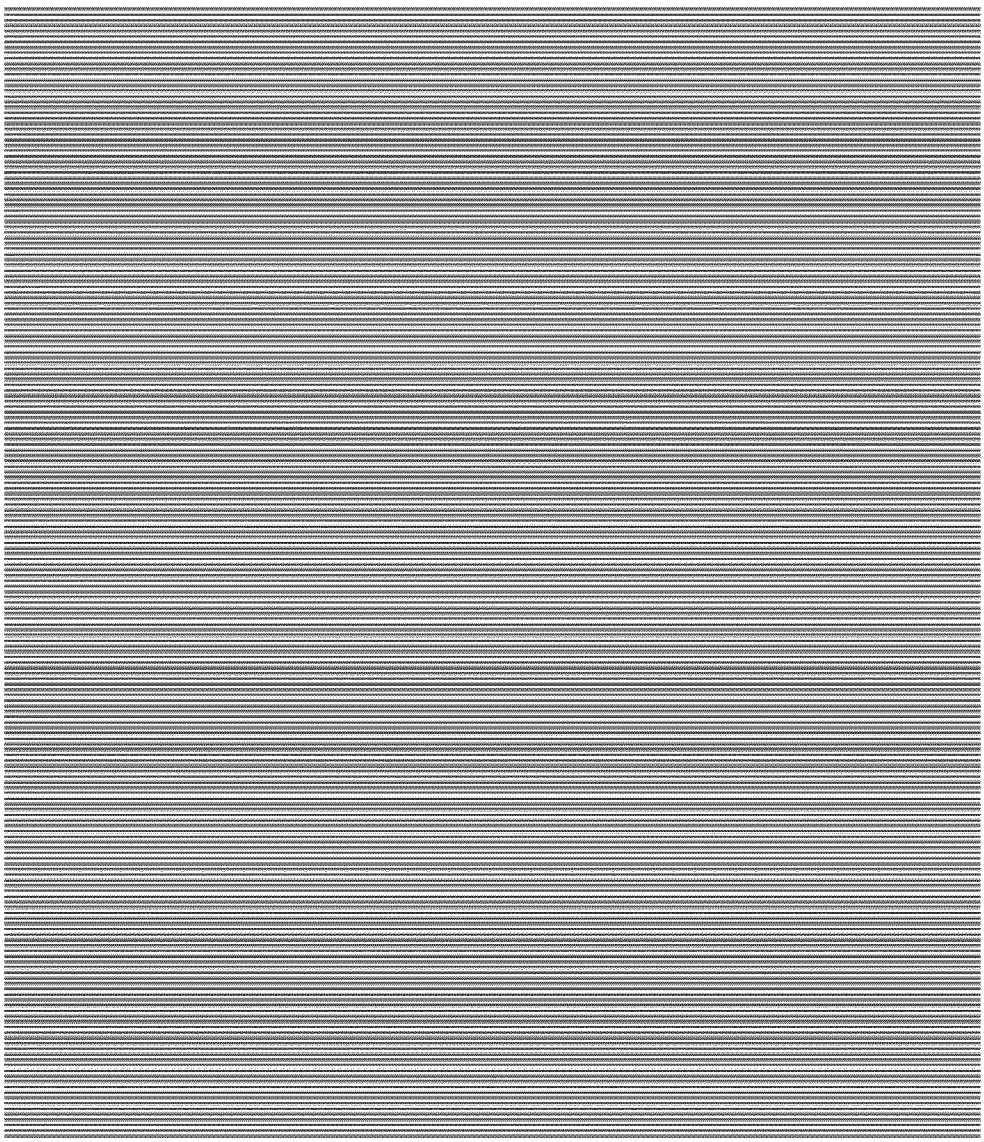

FIG. 7B

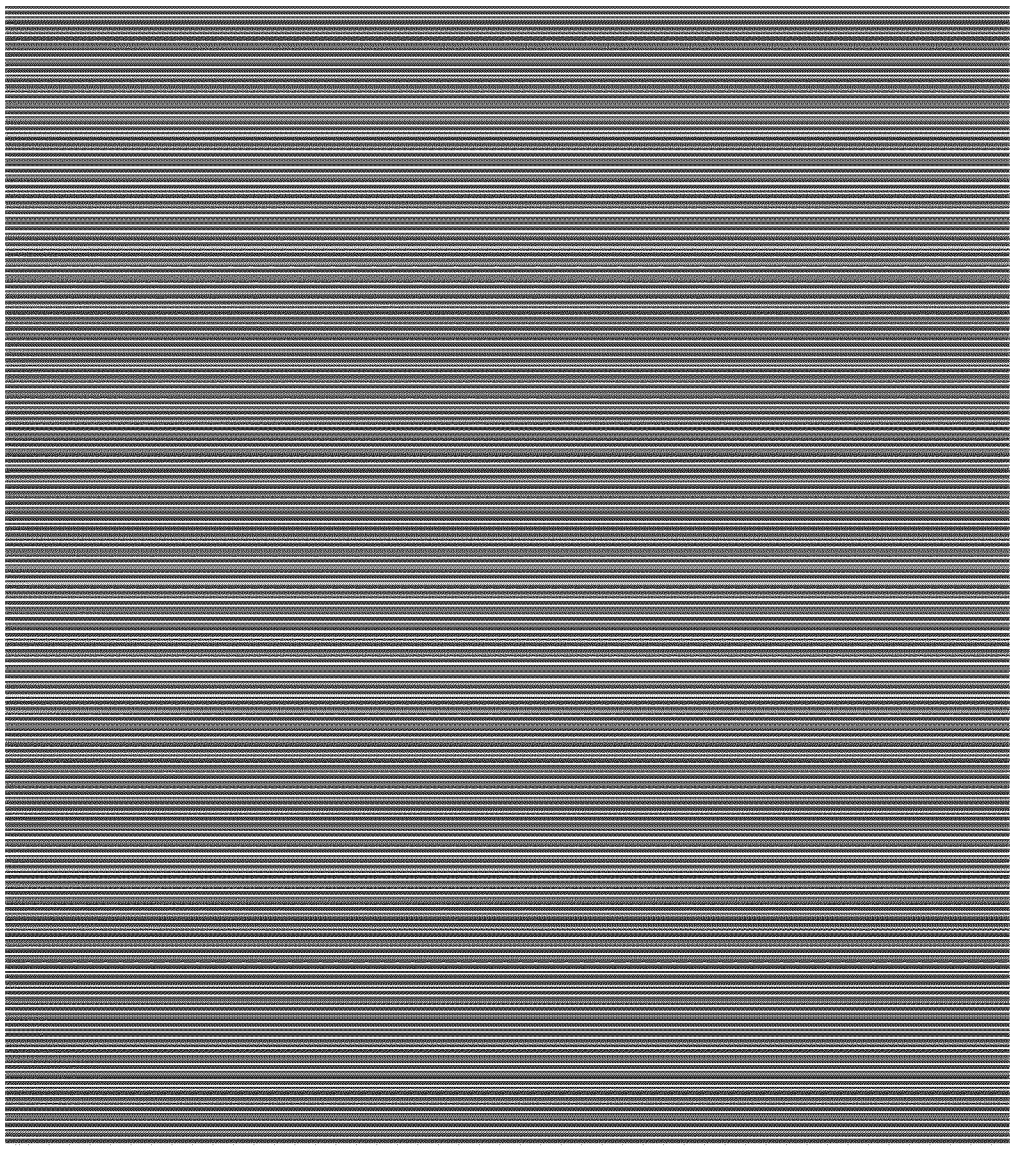

FIG. 7C

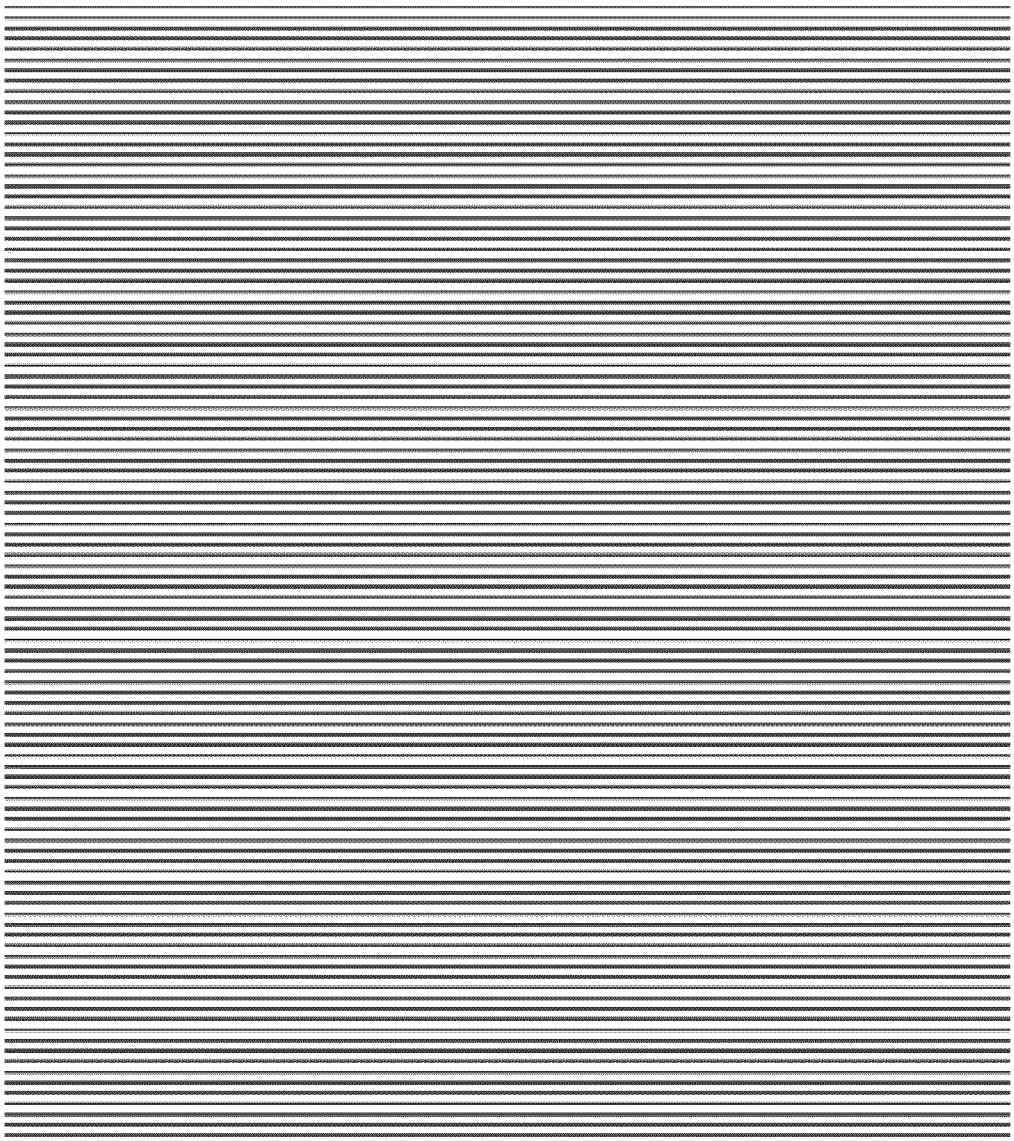

FIG. 7D

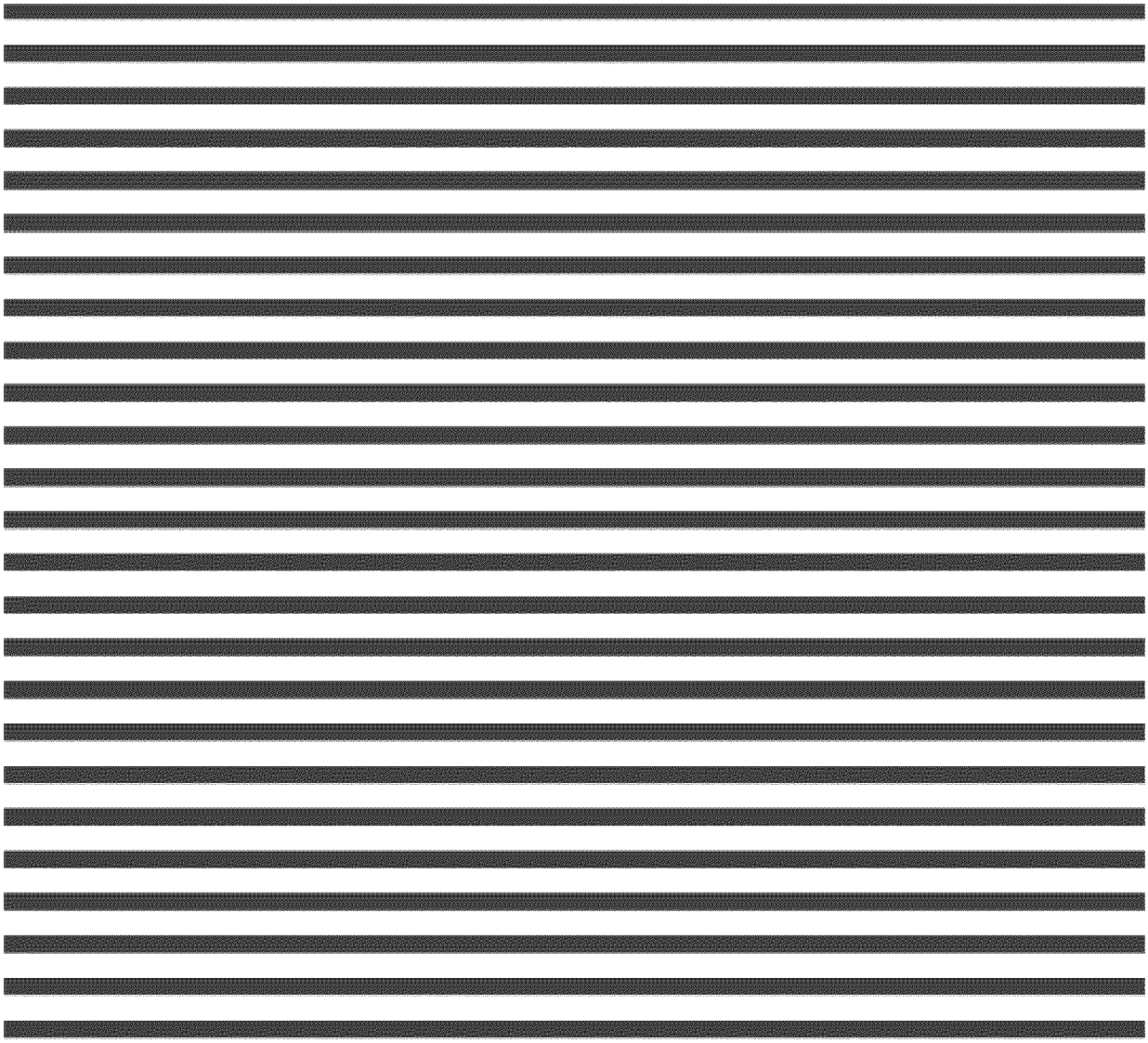

FIG. 7E

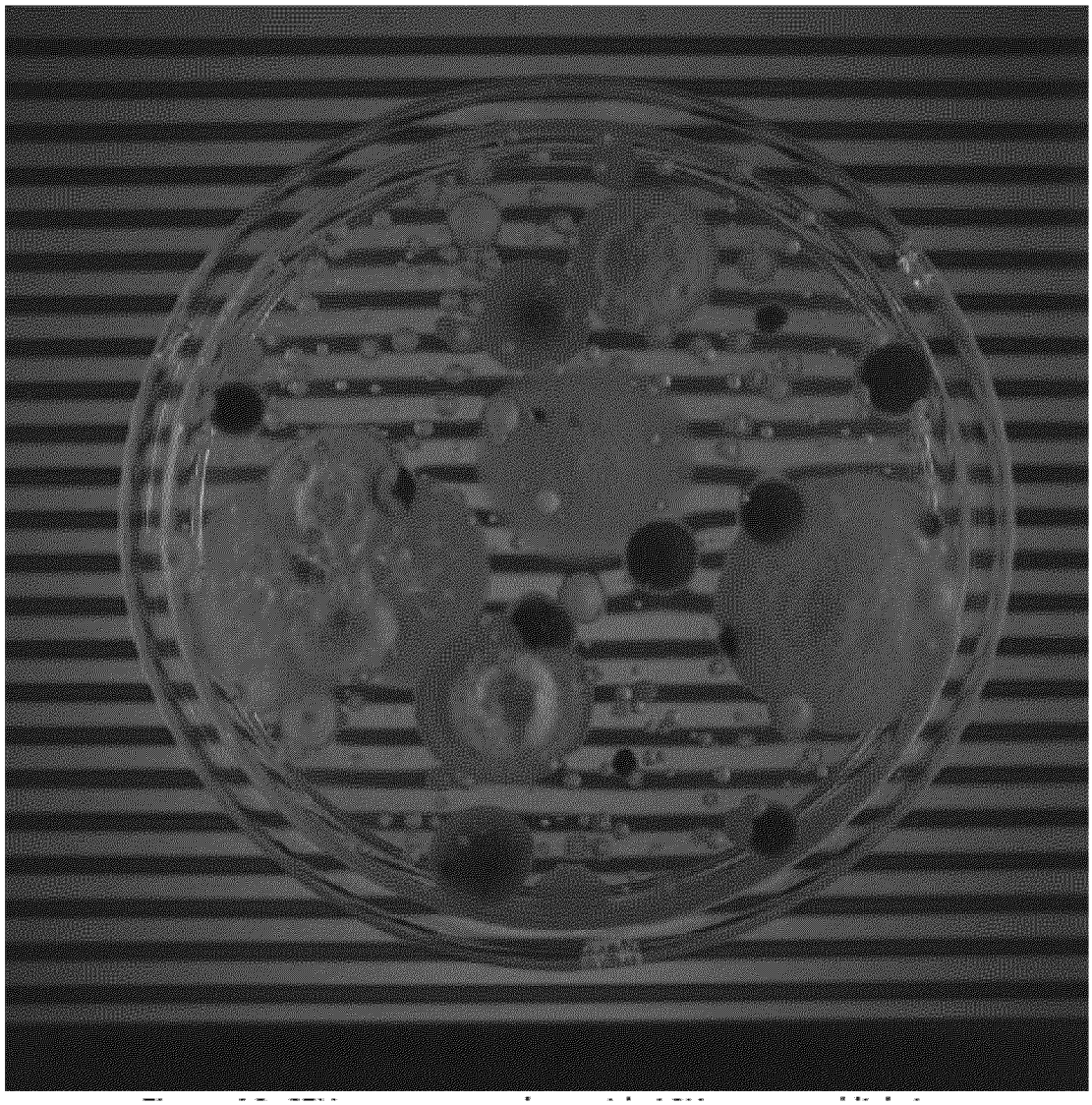

FIG. 7F

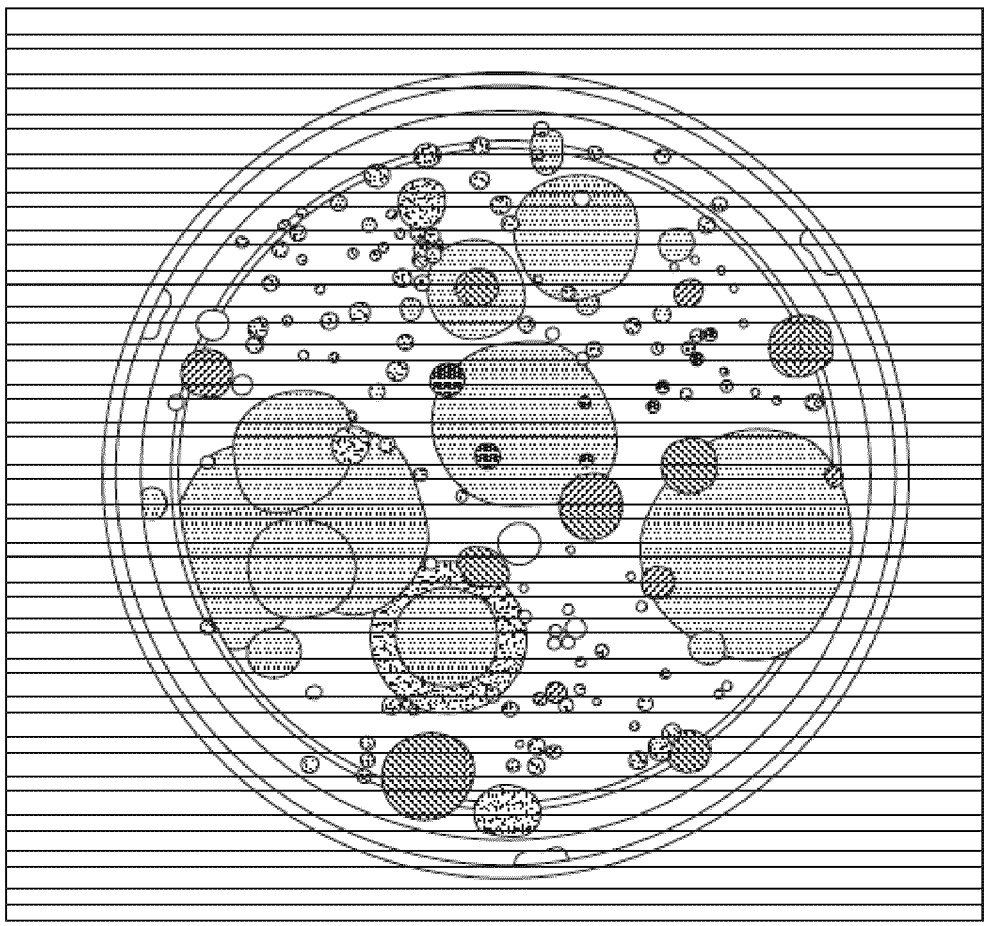

FIG. 7G

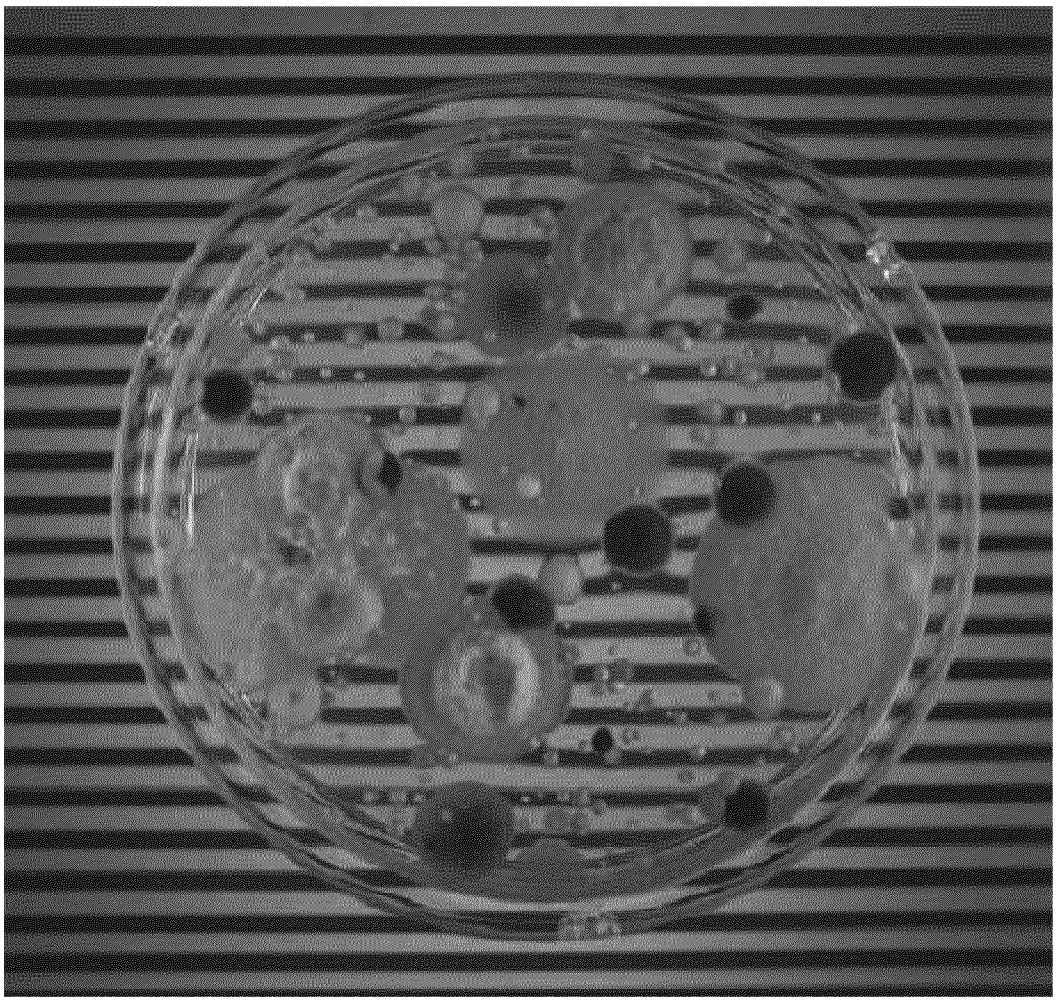

FIG. 7H

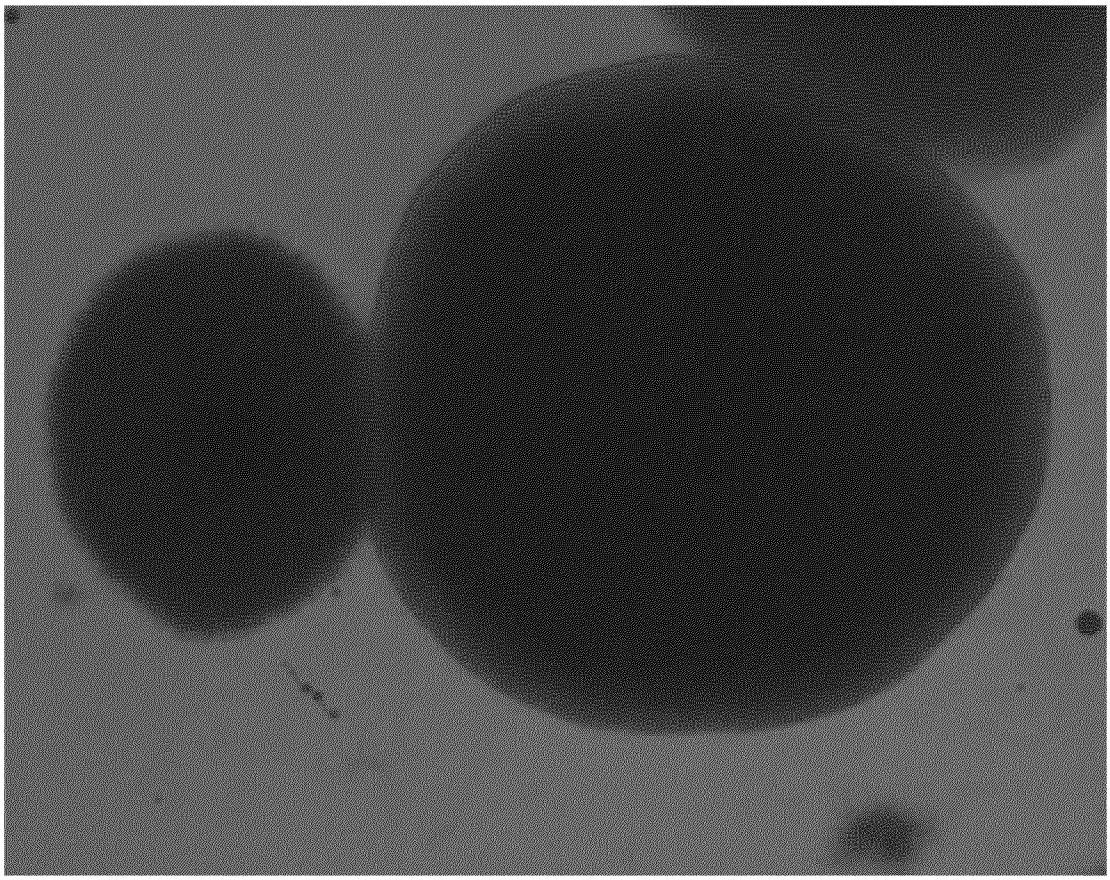

FIG. 8A

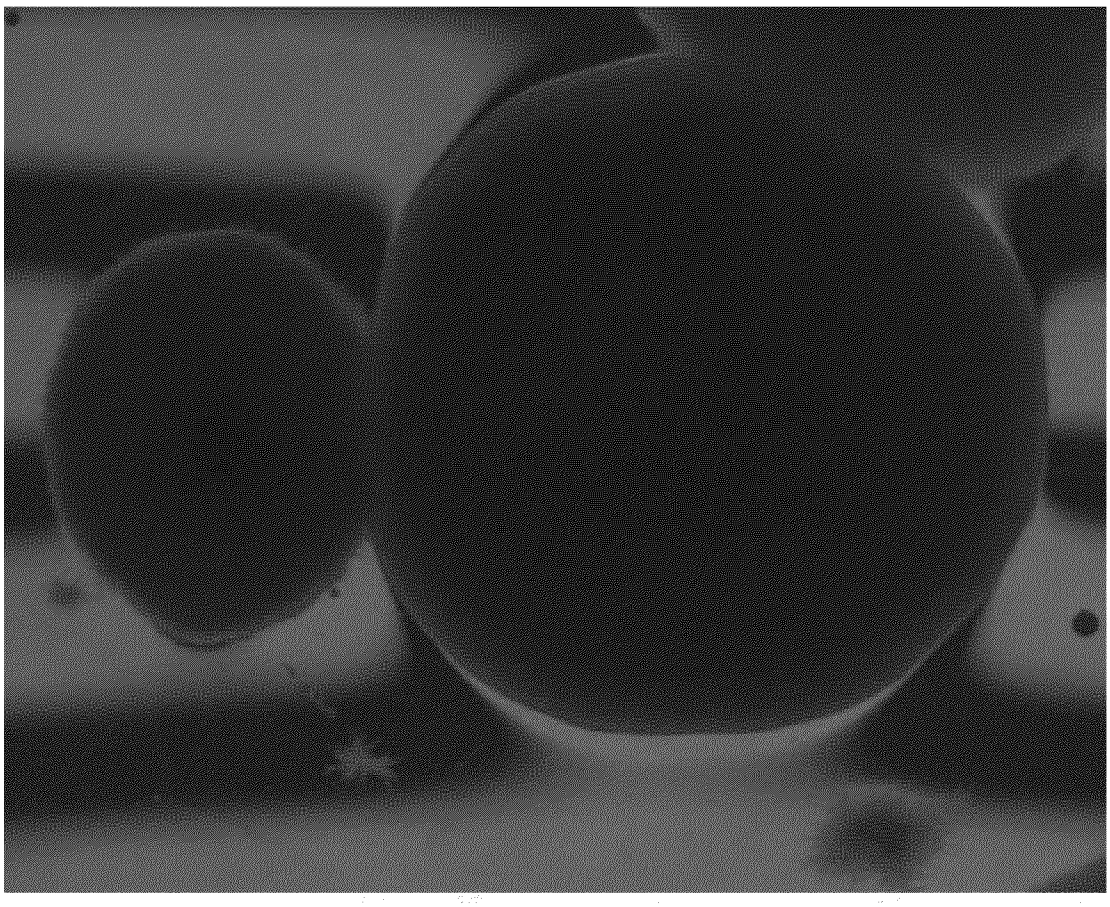

FIG. 8B

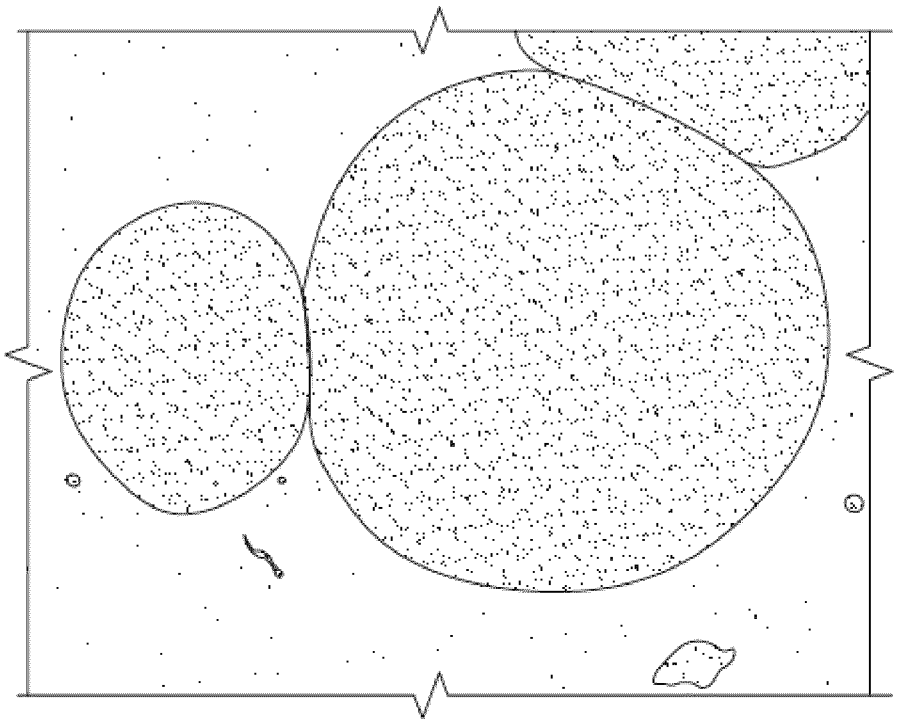

FIG. 8C

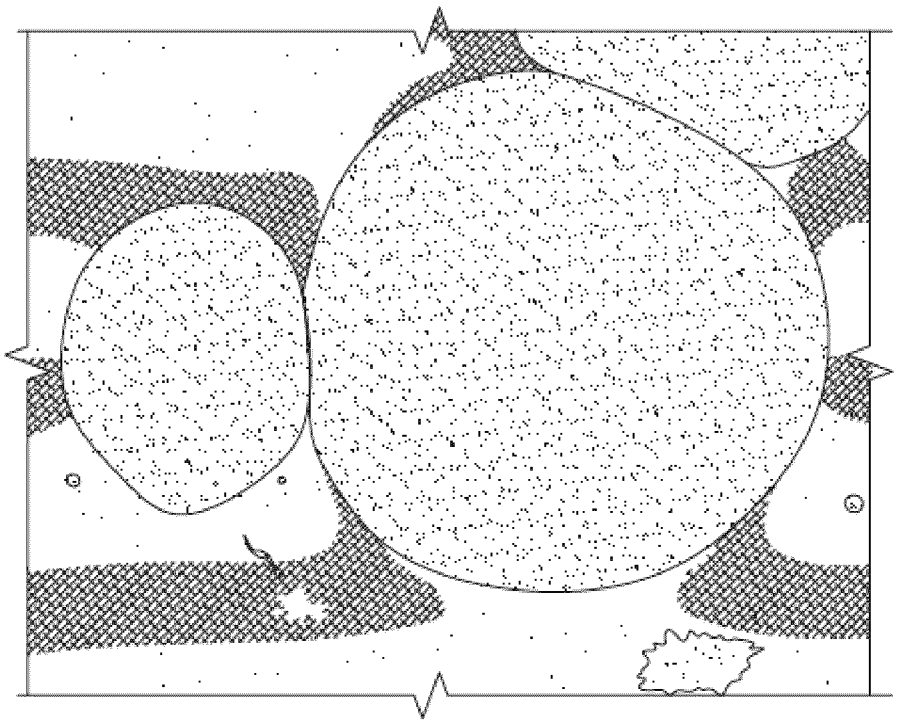

FIG. 8D

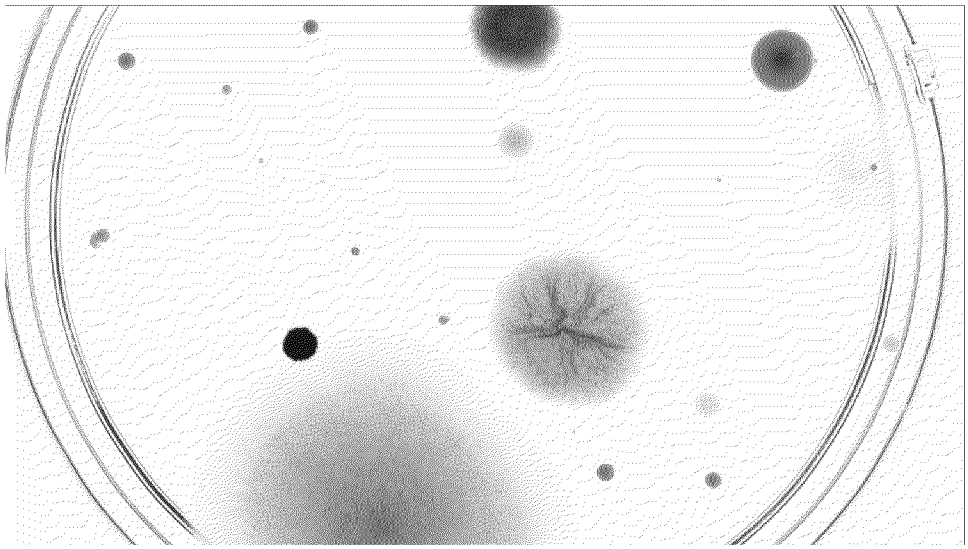

FIG. 9A

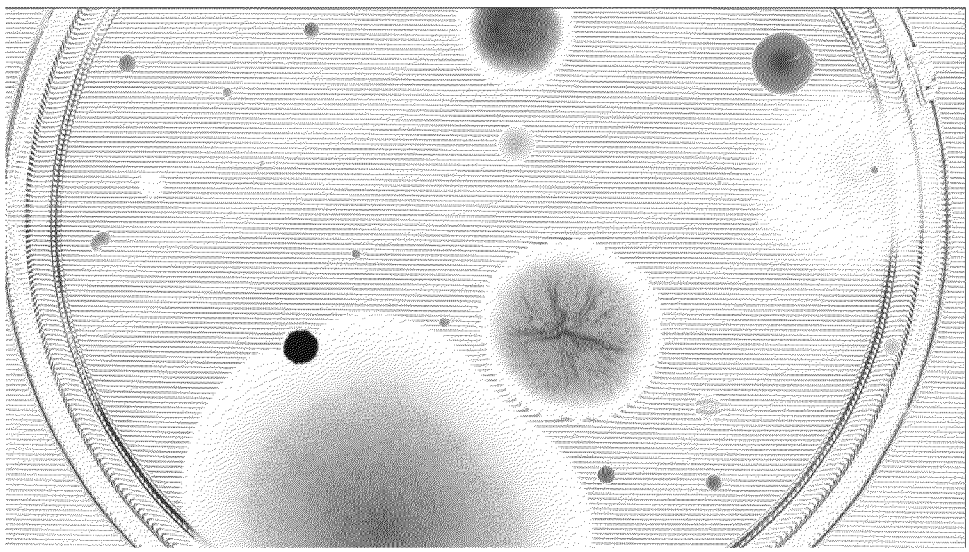

FIG. 9B

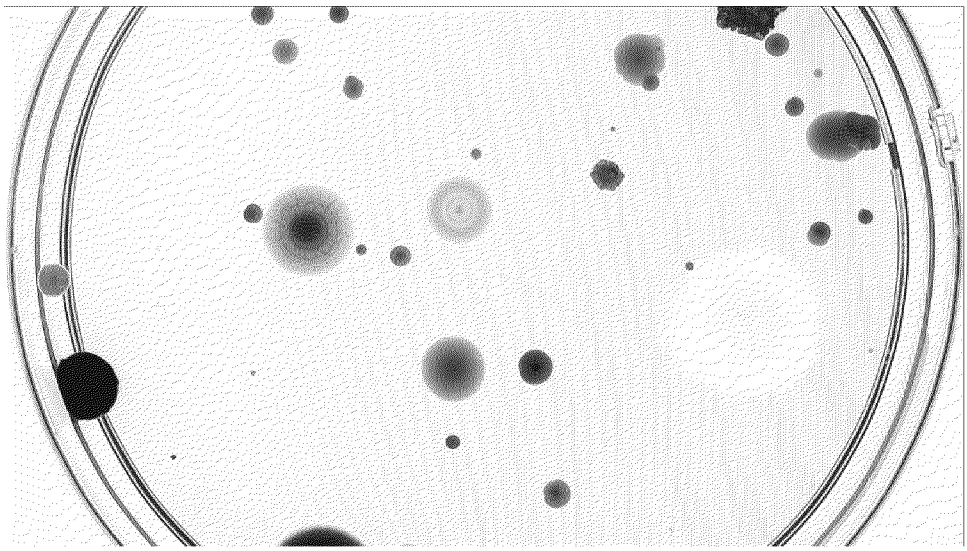

FIG. 10A

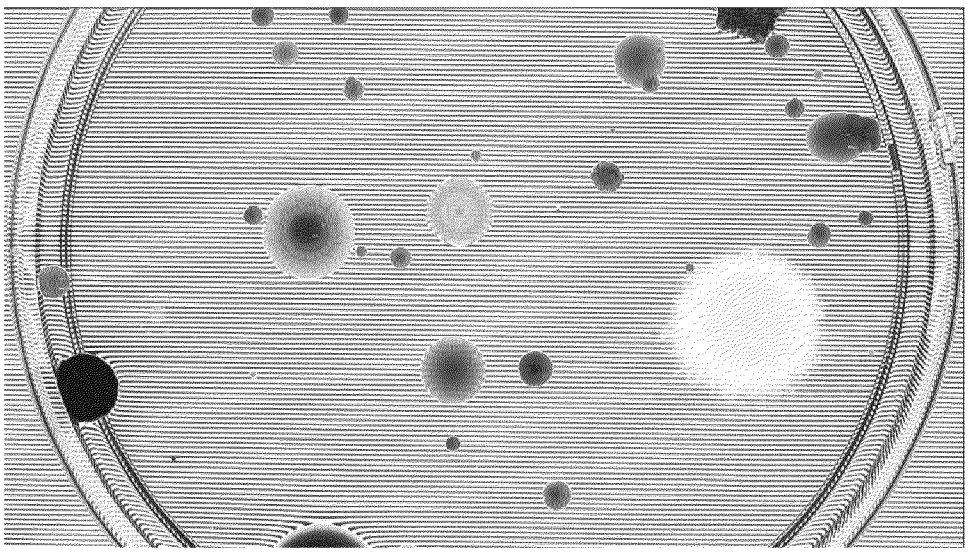

FIG. 10B

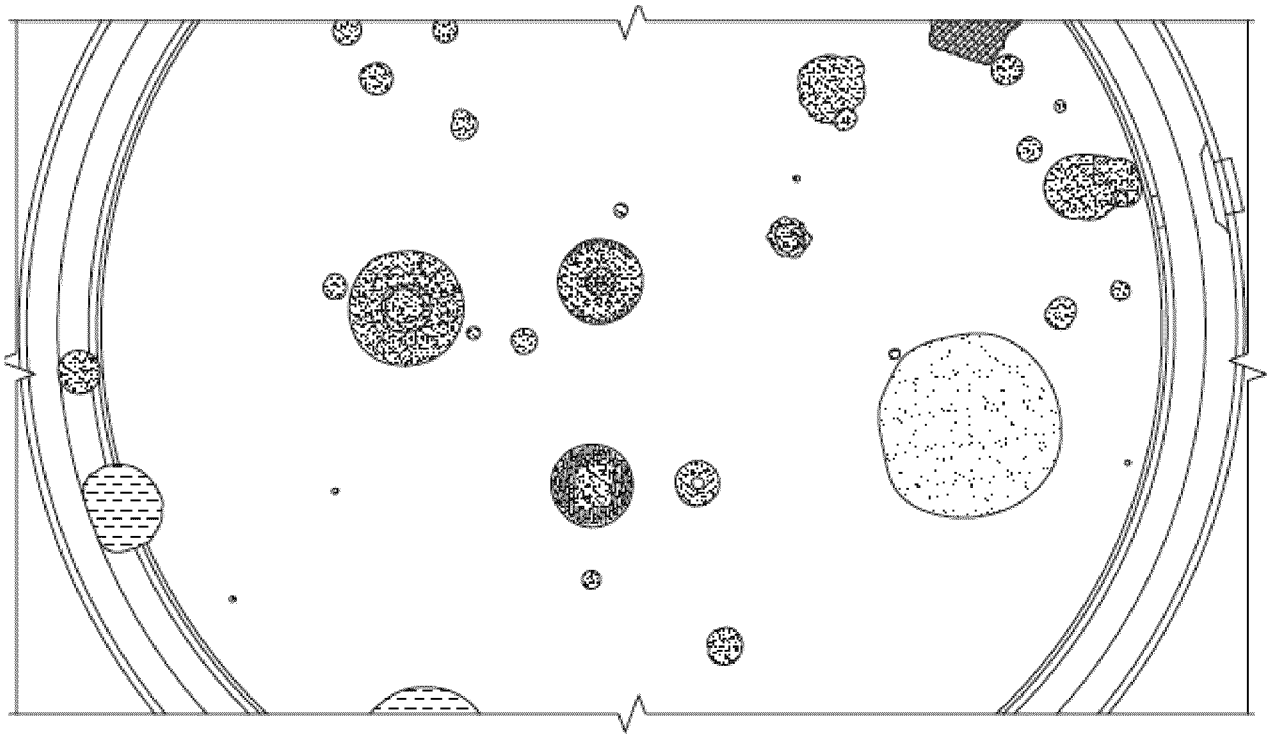

FIG. 10C

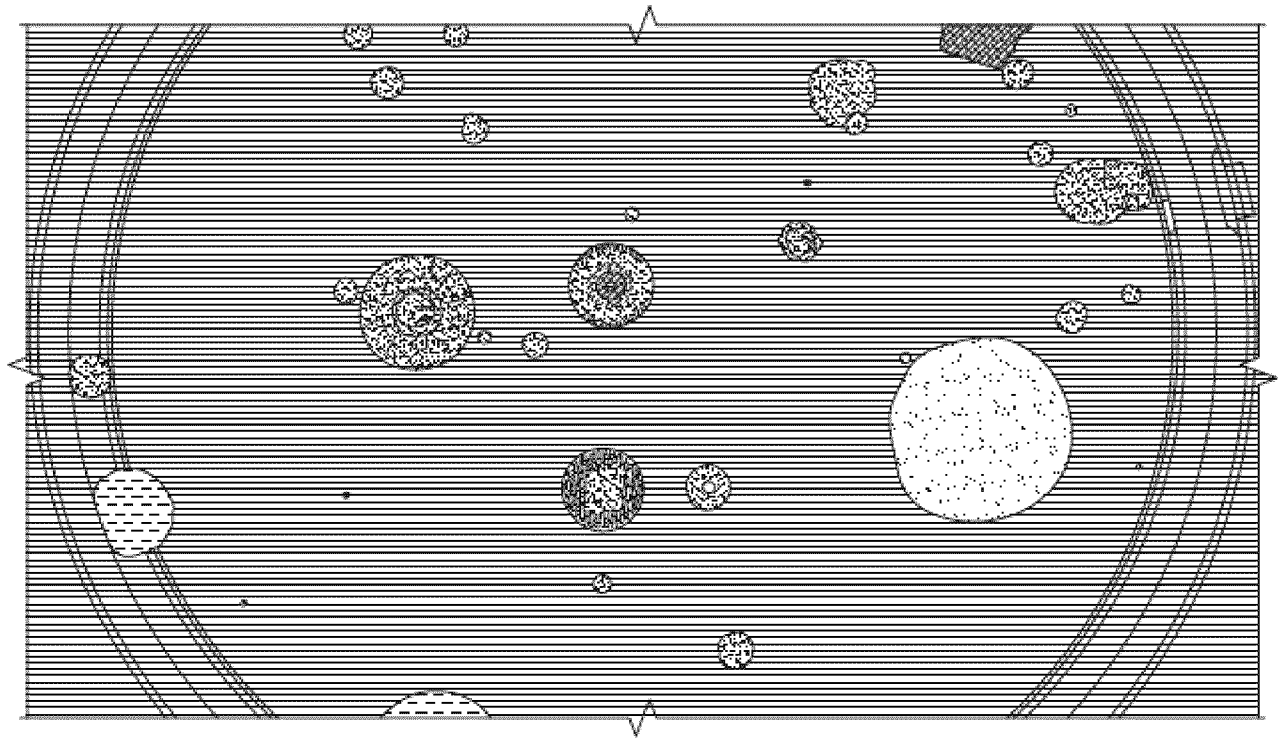

FIG. 10D

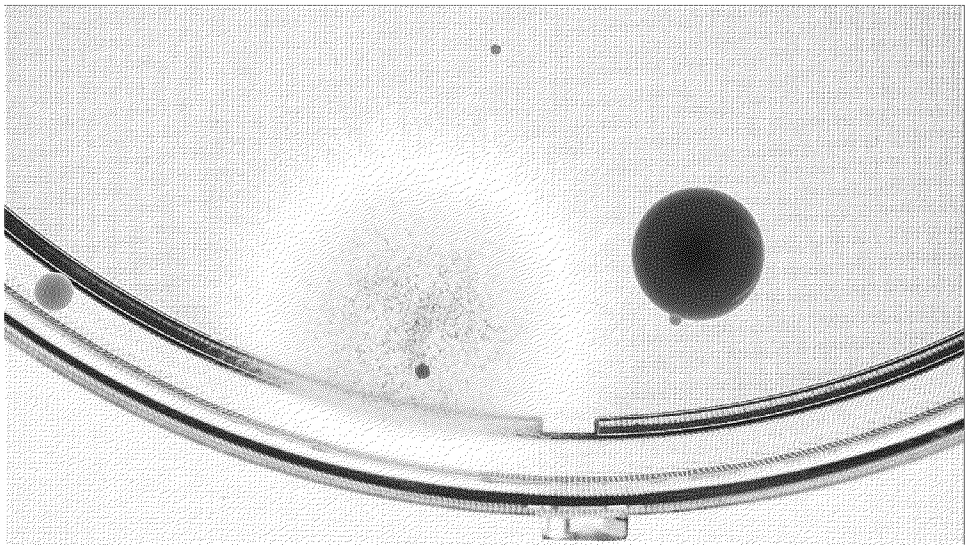

FIG. 10E

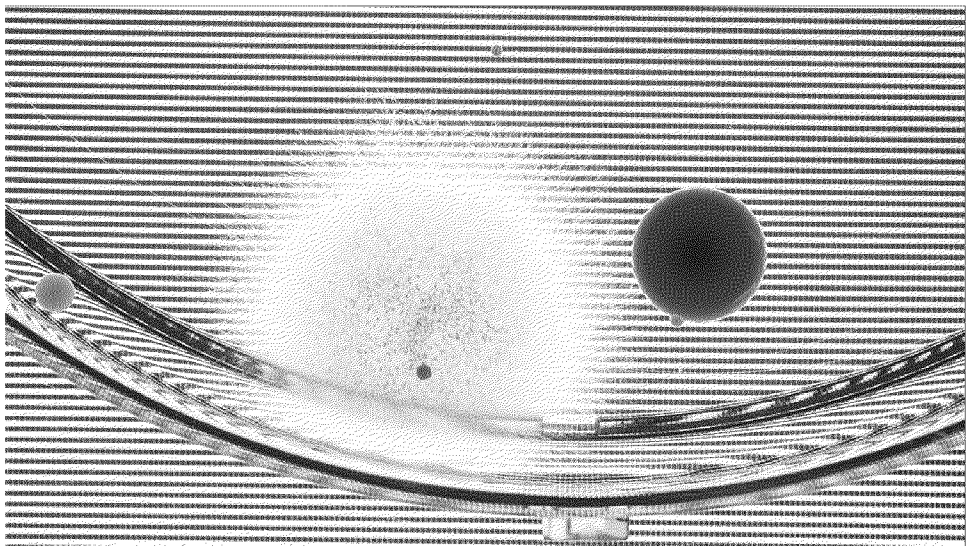

FIG. 10F

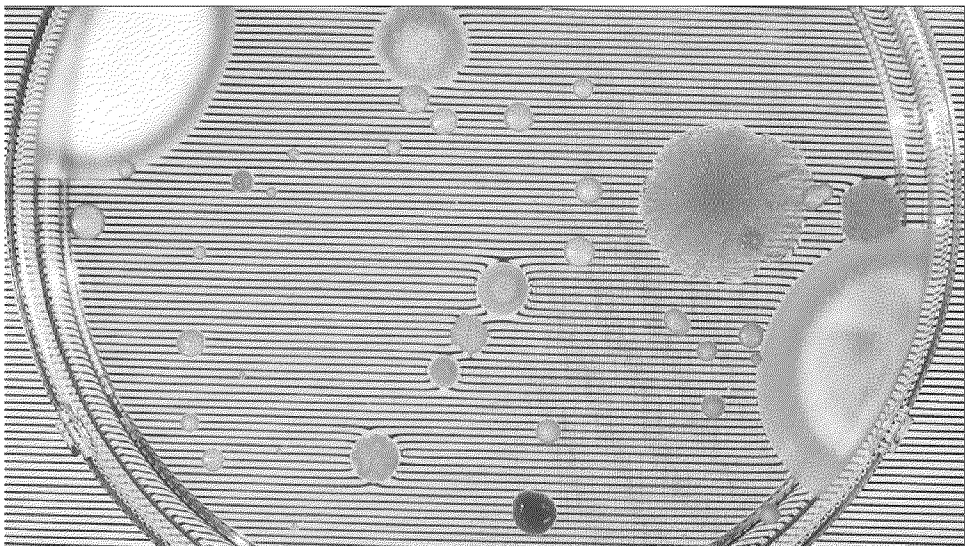

FIG. 11

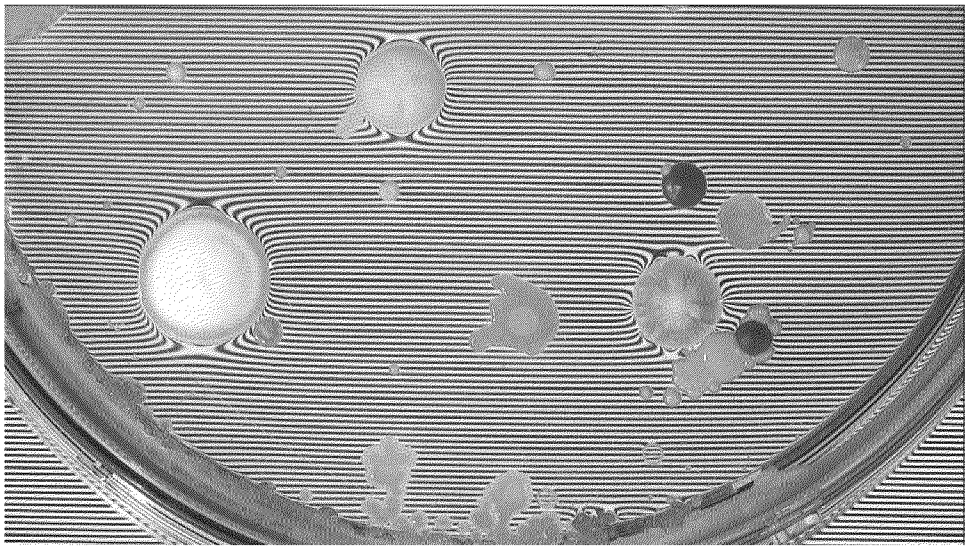

FIG. 12A

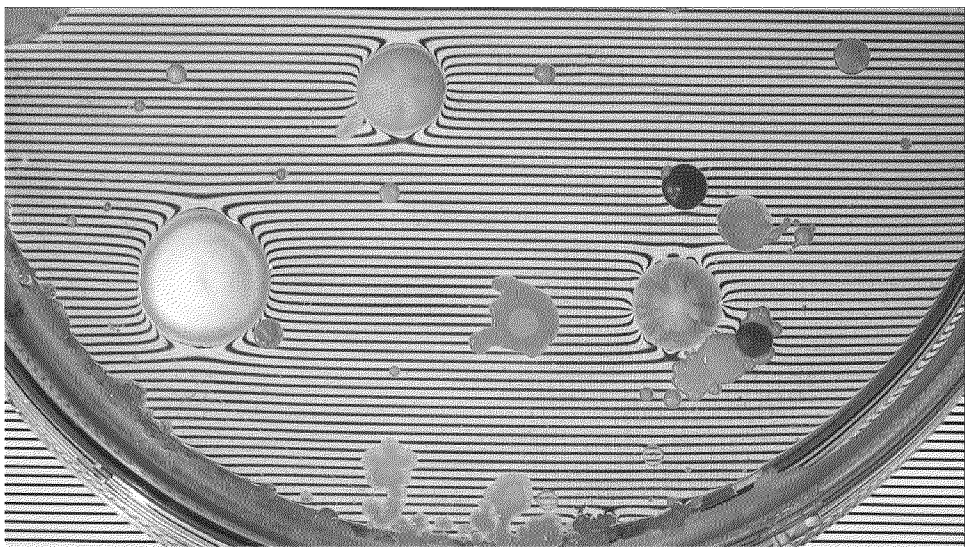

FIG. 12B

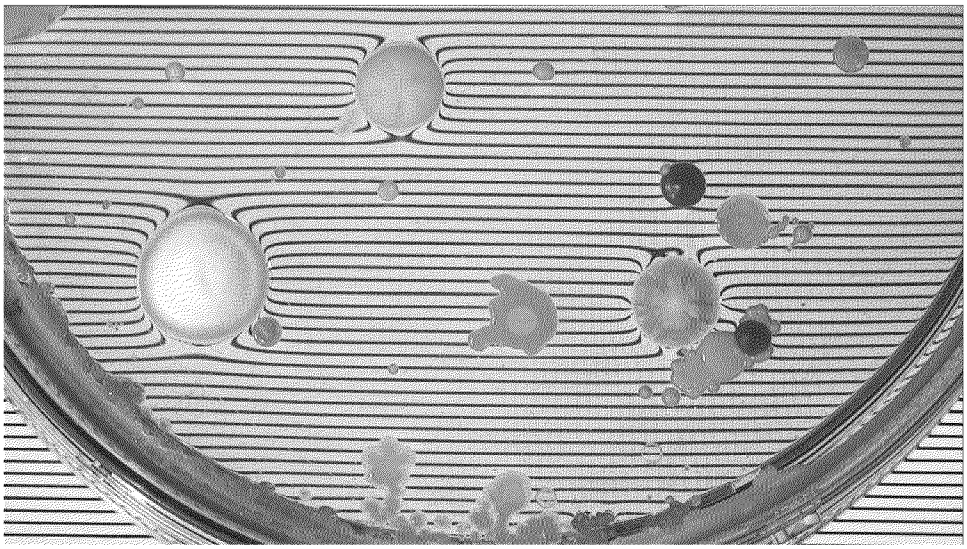

FIG. 12C

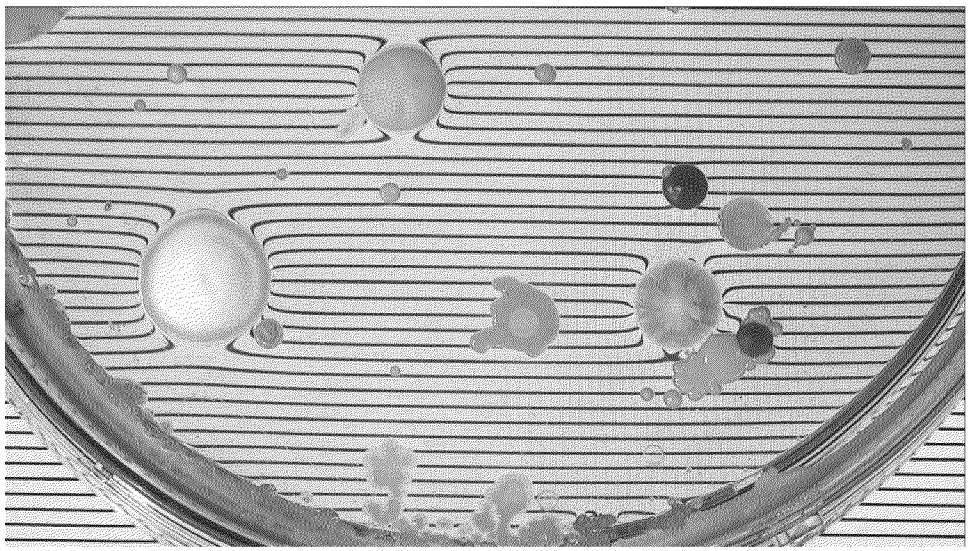

FIG. 12D

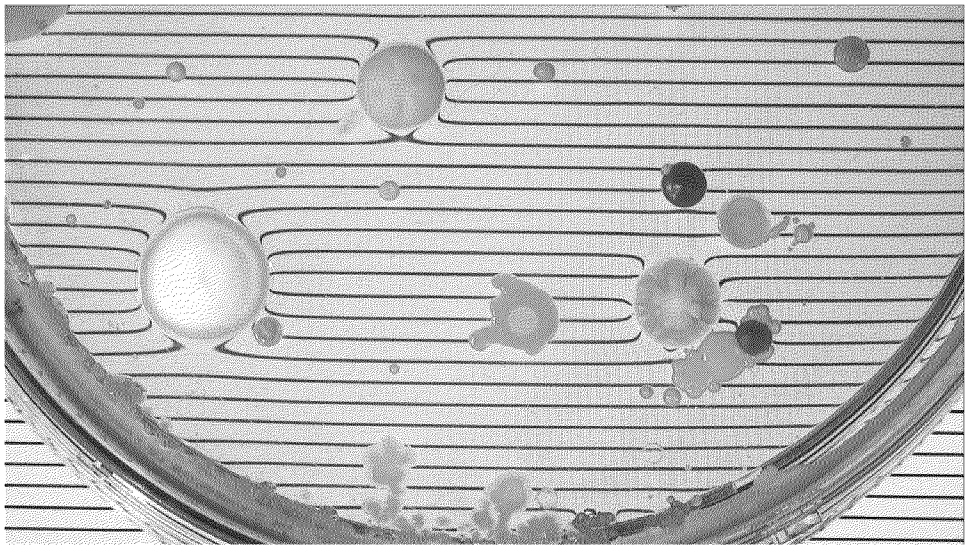

FIG. 12E

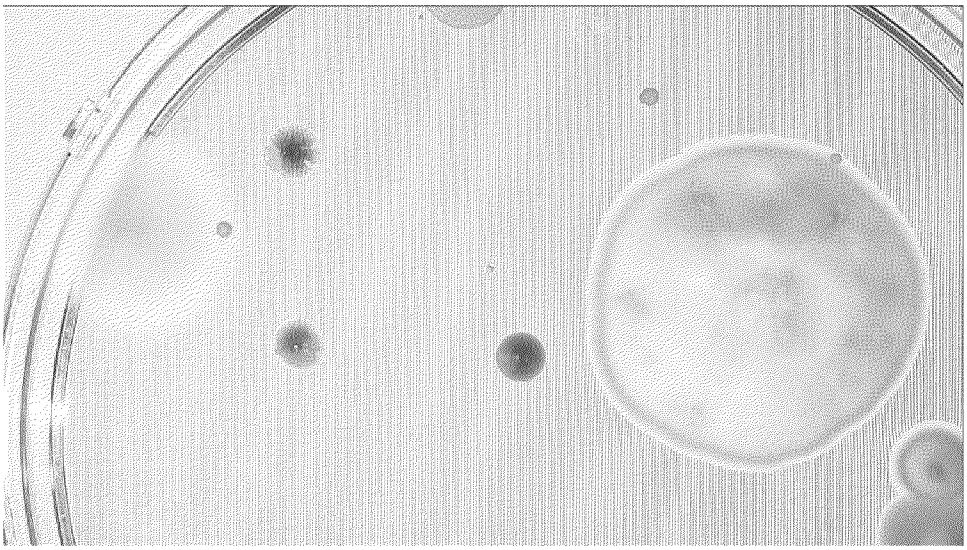

FIG. 13A

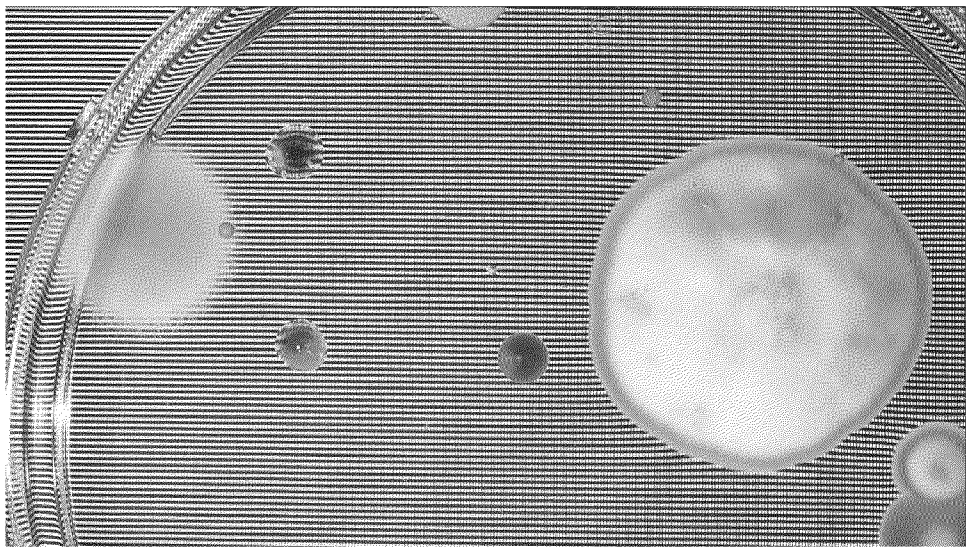

FIG. 13B

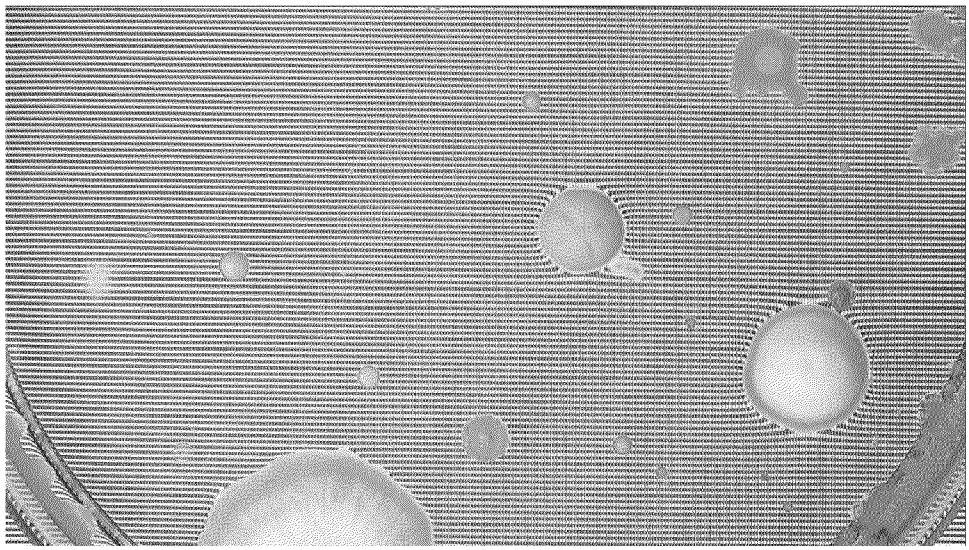

FIG. 14A

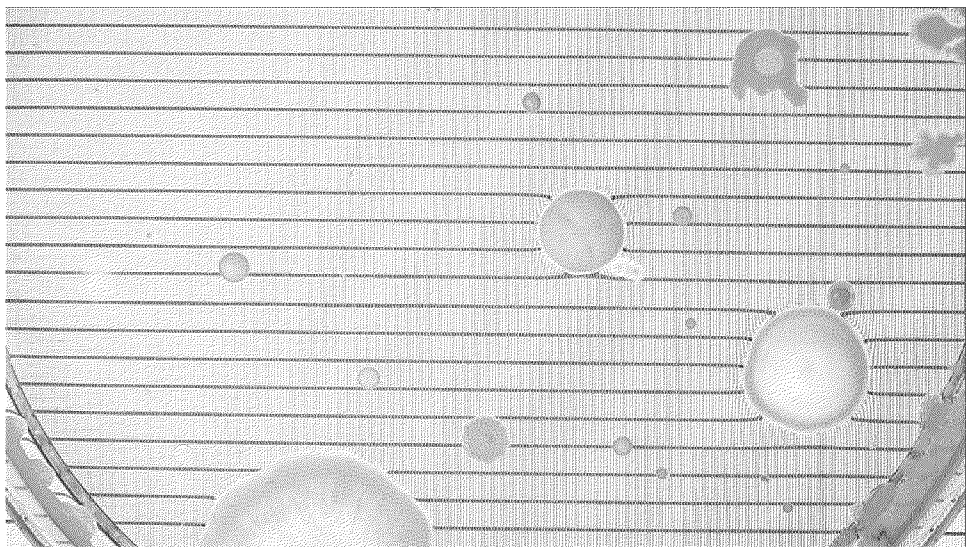

FIG. 14B

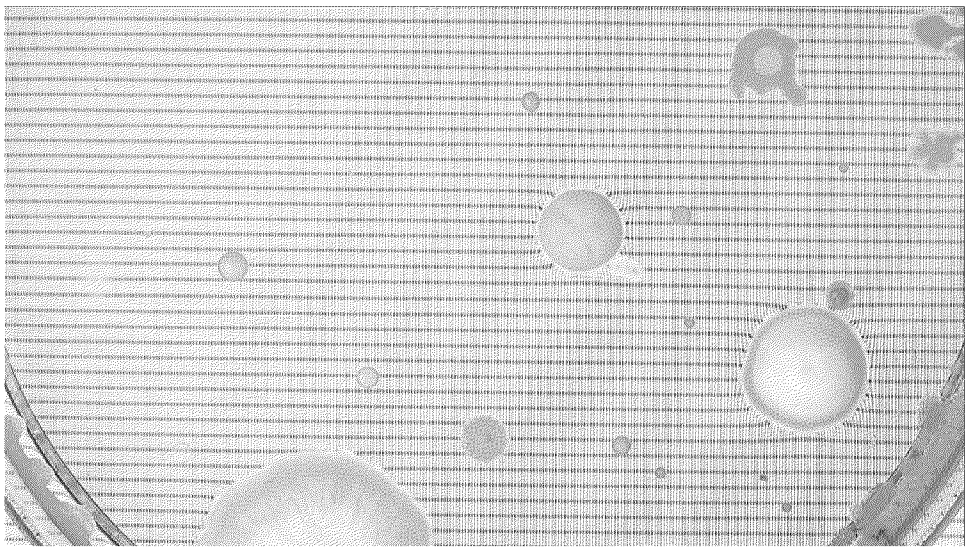

FIG. 14C

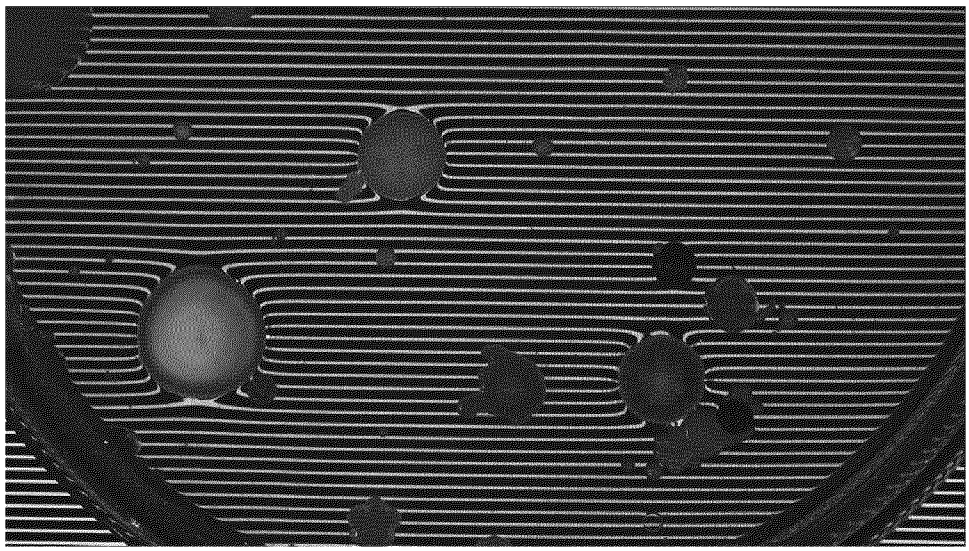

FIG. 15

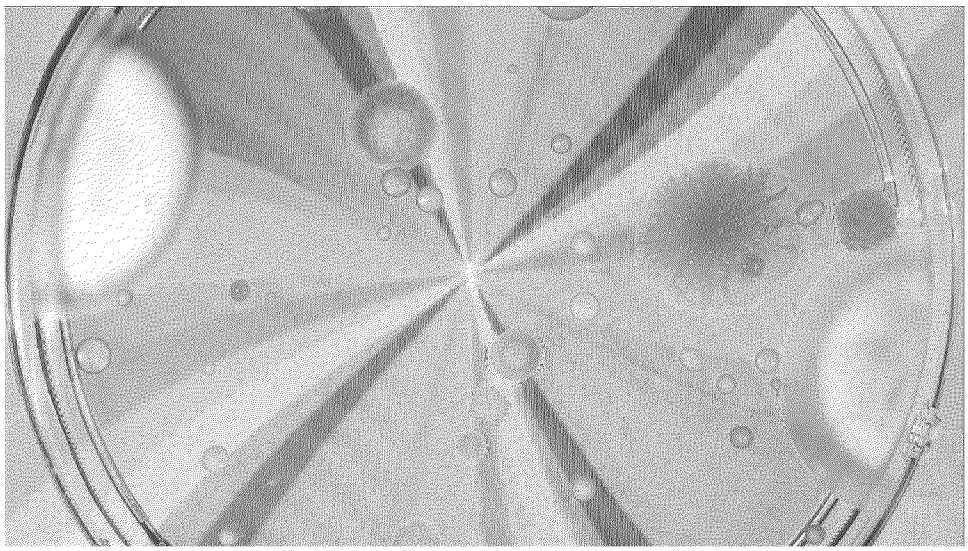

FIG. 16

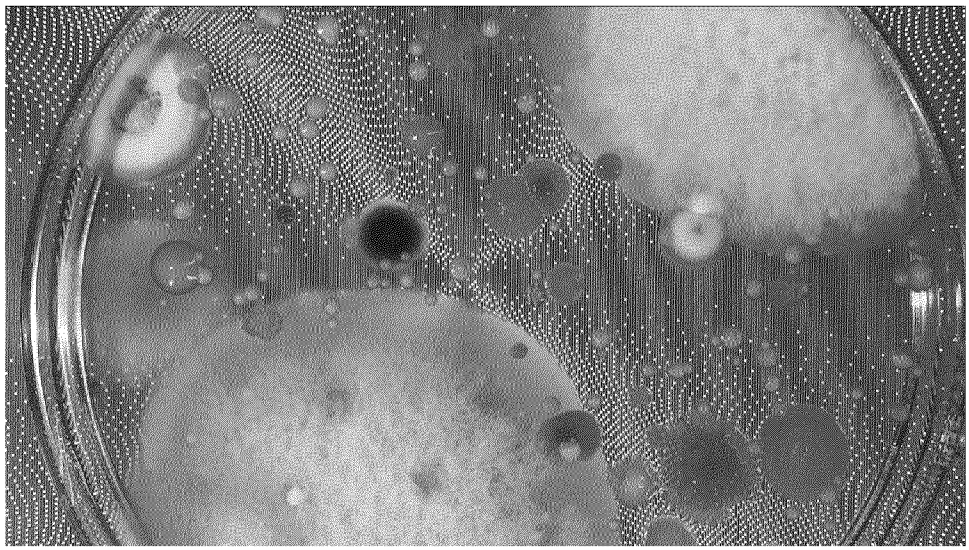

FIG. 17

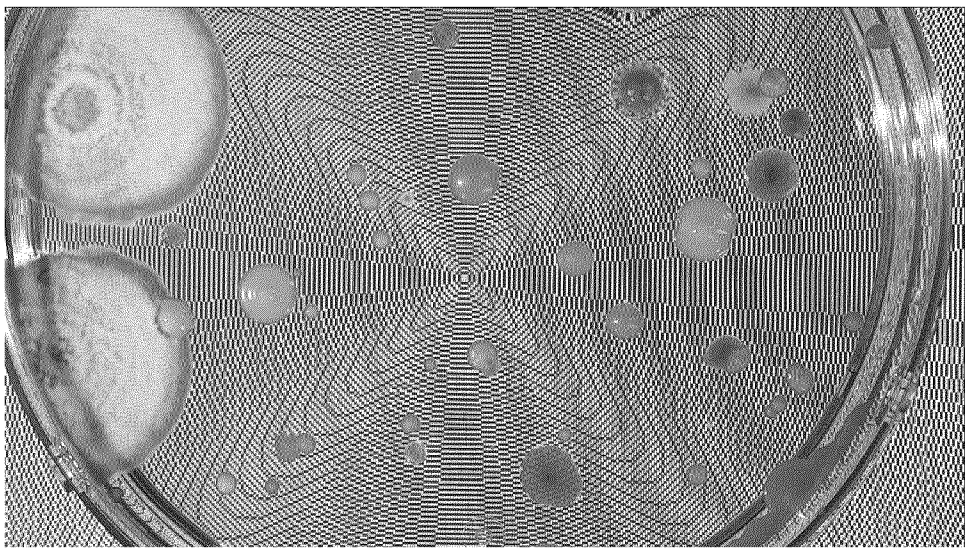

FIG. 18

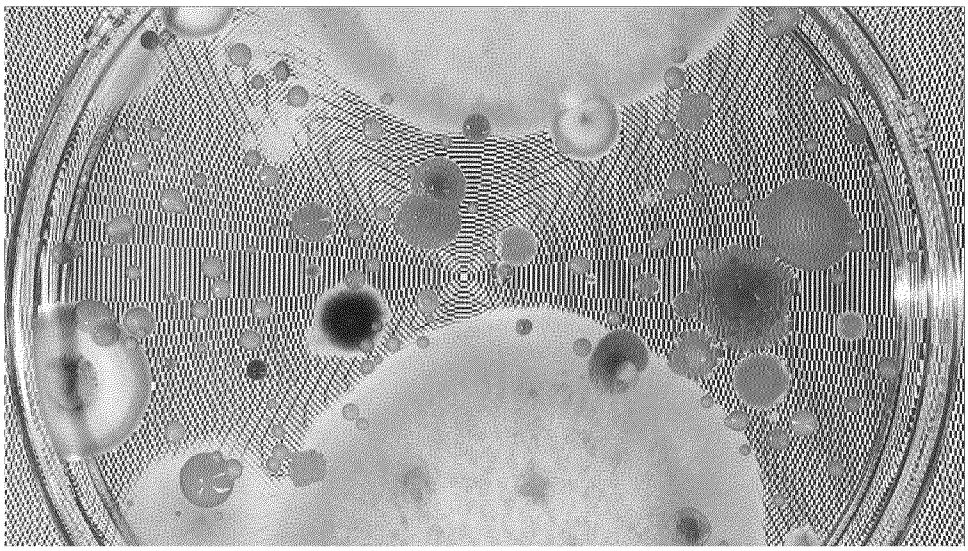

FIG. 19

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/056092

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12M1/34
ADD.

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)
C12M

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/293036 A1 (DDECAUX DOMINIQUE [FR] ET AL) 2 October 2014 (2014-10-02)	1,2,10, 12,13, 17-28
Y	paragraphs [0011], [0024], [0044], [0048] paragraph [0056] - paragraph [0062] paragraph [0108] - paragraph [0111] paragraph [0135] - paragraph [0144] paragraph [0182] - paragraph [0184] paragraph [0222] figures 1-3	3-9,11, 14-16, 29-31

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

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"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 October 2022	31/10/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Cubas Alcaraz, Jose

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/056092

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	US 2012/275681 A1 (HONDA TOSHIFUMI [JP] ET AL) 1 November 2012 (2012-11-01) paragraphs [0001], [0039], [0040] paragraph [0086] - paragraph [0090] paragraphs [0118], [0124], [0147], [0148] paragraph [0199] - paragraph [0203] figures 1,2A,4A,24	1,2,10, 12,13, 17-28
X	US 2014/227774 A1 (GUTHRIE LUSIA HALINA [AU] ET AL) 14 August 2014 (2014-08-14) paragraphs [0011], [0021] paragraph [0025] - paragraph [0033] paragraph [0048] - paragraph [0063] figure 1	1,2,10, 17-28
Y	US 2009/087075 A1 (KII HIROAKI [JP] ET AL) 2 April 2009 (2009-04-02) paragraph [0008] paragraph [0010] - paragraph [0014] paragraph [0030] - paragraph [0046] figures 1A,1B	3-9,11, 14-16, 29-31
A	US 2015/268163 A1 (DUPOY MATHIEU [FR] ET AL) 24 September 2015 (2015-09-24) paragraph [0027] - paragraph [0036]; figures 1,2A,2B,2C	1-31
A	WO 2021/234513 A1 (3M INNOVATIVE PROPERTIES CO [US]) 25 November 2021 (2021-11-25) paragraphs [0003], [0004], [0049], [0050] paragraph [0054] - paragraph [0065] paragraphs [0137], [0139], [0141], [0160] figure 2	1-31

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2022/056092

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