



(12) APPLICATION

(11) **20201084**

(13) **A1**

NORWAY

(19) NO

(51) Int Cl.

G01N 33/48 (2006.01)

G01N 33/483 (2006.01)

G01N 33/487 (2006.01)

A01K 61/00 (2017.01)

A01K 61/17 (2017.01)

B07C 5/34 (2006.01)

B07C 5/342 (2006.01)

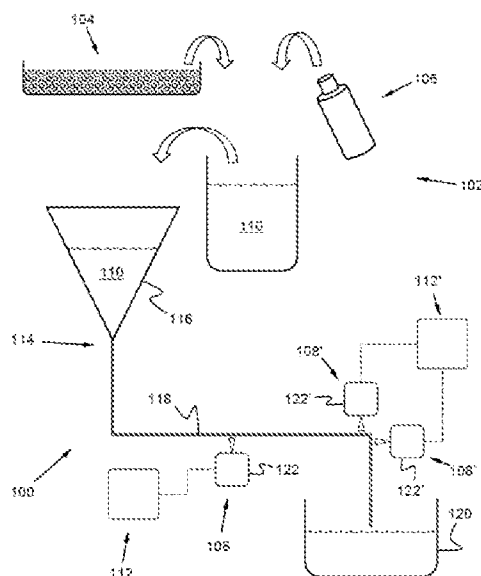
G01B 9/00 (2006.01)

Norwegian Industrial Property Office

(21)	Application nr	20201084	(86)	Int. application day and application nr	
(22)	Application day	2020.10.06	(85)	Entry into national phase	
(24)	Date from which the industrial right has effect	2020.10.06	(30)	Priority	2020.05.15, NO, 20200577
(41)	Available to the public	2021.11.16			
(71)	Applicant	CRYOGENETICS AS, Storhamargata 44, 2317 HAMAR, Norge			
(72)	Inventor	Steffen Wolla, Grønndalsbakken 138, 2407 ELVERUM, Norge			
		Inger Grevle, Måsåbekkvegen 13D, 2316 HAMAR, Norge			
(74)	Agent or Attorney	ONSAGERS AS, Postboks 1813, Vikå, 0123 OSLO, Norge			

(54)	Title	METHOD AND SYSTEM FOR IDENTIFYING FERTILIZED FISH EGGS
(57)	Abstract	

A method of identifying fertilized fish eggs (200) in a sample of eggs (104) taken from a batch of incubating fish eggs is disclosed. The method includes the steps of treating the sample, within a period of time from initial fertilization of the batch, with a fixative (106) bringing an outer membrane of the eggs at least partly transparent and/or a blastodisc of the eggs at least partly opaque. The method further includes the step of capturing at least one image of individual eggs of the sample depicting the blastodisc of the egg, analysing the at least one image for detecting divided cells in the blastodisc, and identifying each egg associated with an image showing divided cells in the blastodisc as a fertilized egg. An associated system and a fish egg analysis apparatus are also disclosed.



METHOD AND SYSTEM FOR IDENTIFYING FERTILIZED FISH EGGS

The present invention relates to a method of analysing fish eggs with the purpose of determining whether they are fertilized. The present invention also relates to an associated system.

- 5 The present invention also relates to an apparatus capable of analysing fish eggs with the purpose of determining whether they are fertilized.

In aquaculture fish farming, spermatozoa and eggs are mixed to allow the eggs to be impregnated and fertilized. In order to determine the success rate of the fertilization process, a sample of eggs may be drawn from the fertilized batch and analysed to determine the ratio
10 between the number of fertilized eggs and the number of unfertilized ones, e.g. the fertilization rate.

In teleost fish, a blastodisc located at an animal pole of the egg, close to an outer membrane of the egg, is formed shortly after fertilization, in which disc initial cell division, or cleavage, takes place. In some fish species, the outer membrane of the egg is opaque, thus making it
15 difficult to detect early cleavage. Such species include species in the family Salmonidae, e.g. the Atlantic salmon (*Salmo salar*).

One object of the present disclosure is to address this problem. Another object is to provide a useful method and an associated method of analysing fish eggs, especially with the purpose of determining whether the eggs are fertilized. A further object is to provide a beneficial
20 apparatus capable of analysing fish eggs.

According to one aspect, the present disclosure provides a method of identifying fertilized fish eggs in a sample of eggs taken from a batch of incubating fish eggs. The method comprises the steps of:

- within a period of time from initial fertilization of the batch, treating the sample with a
25 fixative bringing an outer membrane of the eggs at least partly transparent and/or a blastodisc of the eggs at least partly opaque;
- capturing images of individual eggs of the sample depicting the blastodisc of the egg;
- analysing the images for detecting divided cells in the blastodisc; and
- identifying each egg associated with an image showing divided cells in the blastodisc as
30 a fertilized egg.

The step of capturing images of individual eggs may comprise capturing a plurality of images of each egg from a plurality of directions, i.e. from different directions. The step of capturing images of individual eggs may comprise capturing, in a single exposure, a plurality of images of each egg from a plurality of directions. One or a plurality of prisms and/or mirrors may
35 be used to capture, in said single exposure, said images of the egg from said plurality of directions.

The step of capturing images of individual eggs may comprise bringing the eggs in single file passed an imaging system, i.e. guiding the eggs one-by-one through the imaging system.

The period of time from initial fertilization of the batch to treating the sample with the fixative may be within the range of 30 to 220 degree-hours. Degree-hours is a measure which takes into account the temperature in the incubator when timing fish egg development. In particular, degree-hours divided by the temperature in the incubator (in centigrades) will give a time measure. For example, if the temperature in the incubator is 10°C, the sample should preferably be treated with the fixative within the range of 30 degree-hours/10°C to 220 degree-hours/10°C = 3 to 22 hours post fertilisation. For most fish species this will give the eggs sufficient time to develop detectable cleavage in the blastodisc, e.g. 2 to 16 cells. When the sample is to be treated with the fixative may vary between species. For example, eggs of the species *S. salar* may advantageously be treated with the fixative within the range of 170 to 190 degree-hours, e.g. at 180 degree-hours.

The fixative may comprise acetic acid and may advantageously comprise any one of an acetic acid-alcohol-formaldehyde solution, such as Serra's fixative or Stockard's solution, or a non-formaldehyde acetic acid solution, such as the fixative marketed under the trade name AquaBoost® Quattro.

The method may comprise the step of compressing the egg to a predetermined thickness prior to said step of capturing said at least one image of the egg. The predefined thickness may be any one of: within the range of 0.1 to 1.5 mm; within the range of 0.3 to 1.0 mm; within the range of 0.4 to 0.6 mm; and 0.5 mm. At least for eggs of the species *S. salar*, this may be an advantageous predefined thickness.

The step of compressing the egg may comprise the sub-steps of trapping the egg between two holding elements and bringing the holding elements together compressing the trapped egg. The holding elements may comprise ductile strips of transparent sheet material. The transparent sheet material may comprise regenerated cellulose, e.g. CELLOPHANE (TM).

The step of capturing an image of the compressed egg may comprise the sub-steps of: arranging an image-capturing device on a first side of the compressed egg; arranging a light source on a second side of the compressed egg, said second side being opposite the first side; and transmitting light from the light source (427, 627) through the compressed egg and into the image-capturing device.

According to another aspect, the present disclosure provides a fish egg analysis system comprising:

- at least one imaging system being configured for capturing at least one image of individual fish eggs of a sample of eggs comprising fertilized and unfertilized fish eggs depicting the blastodisc of the eggs;

- an image analysis system being configured for analysing the captured images for detecting divided cells in the blastodisc and identifying each egg associated with an image showing divided cells in the blastodisc as a fertilized egg; and
- a conveyor system arranged for conveying the eggs of the sample past the at least one imaging system in single file.

The fish egg analysis system may comprise a fixation system configured for mixing a sample of eggs comprising fertilized and unfertilized fish eggs with a fixative bringing an outer membrane of the eggs at least partly transparent and/or a blastodisc of the eggs at least partly opaque.

- 10 The at least one imaging system may comprise first and second imaging systems being positioned one after the other along conveyor system.

The conveyor system may comprise a tubular conduit for conveying the eggs. The conduit may have an inner diameter D within the range of $D_{\text{egg}} < D < 2 \cdot D_{\text{egg}}$, where D_{egg} is the diameter of the eggs of the sample.

- 15 It has been found that such a system is suited for conveying eggs from species in the family Salmonidae. Eggs of the species *S. salar* normally have a diameter of 6-8.5 mm and the tubular conduit in a system for analysing *S. salar* eggs may have an inner diameter of approximately 10 mm. Eggs of the species *Salmo trutta* (brown trout) normally have a diameter of 4.2 to 5.8 mm and, consequently, the tubular conduit in a system for analysing
- 20 *S. trutta* eggs may have an inner diameter of approximately 6 to 7 mm.

The at least one imaging system may be configured for capturing images of individual eggs from a plurality of directions and the imaging system or systems may comprise one or a plurality of mirrors or prism allowing a camera of the imaging system to capture, in a single exposure, a plurality of images of each egg from different directions.

- 25 The fish egg analysis system may comprise a pressing device arranged upstream of said at least one imaging system, said pressing device being configured to compress each egg to a predetermined thickness. The predetermined thickness may, at least for eggs of the species *S. salar*, be any one of: within the range of 0.1 to 1.5 mm; within the range of 0.3 to 1.0 mm; within the range of 0.4 to 0.6 mm; and 0.5 mm.

- 30 To determine whether an image depicts a blastodisc having divided cells, the image analysis system may employ an algorithm built by a machine learning system utilising mathematical models based on sample or training images of eggs known to be fertilized.

According to yet another aspect, the present disclosure provides a method of determining a fertilization rate of a batch of mixed fish spermatozoa and eggs, the method comprising the

35 steps of:

- drawing a sample comprising a plurality of eggs from the batch;

- within a period of time from initial mixing of the spermatozoa and eggs, treating the sample with a fixative bringing an outer membrane of the eggs of the sample at least partly transparent and/or a blastodisc of the eggs at least partly opaque;
- capturing at least one image of individual eggs of the sample depicting the blastodisc of the egg;
- analysing the images with the purpose of detecting divided cells in the blastodisc;
- classifying each egg associated with an image showing divided cells in the blastodisc as a fertilized egg; and
- calculating the fertilization rate as a ratio between the number of eggs classified as being fertilized and the total number of eggs in the sample.

According to a further aspect, the present disclosure provides a fish egg analysis apparatus comprising:

- an imaging system configured for capturing images of fish eggs;
- an image analysis system configured for analysing the images captured by the imaging system;
- a conveyor system arranged for guiding the eggs through the imaging system allowing the imaging system to capture at least one image of each individual egg; and
- a pressing device configured to compress each fish egg to a predetermined thickness prior to the conveyor system guiding the egg through the imaging system.

The apparatus may comprise a receptacle for receiving a sample of fish eggs, e.g. taken from a batch of fertilized fish eggs, and a conveyor system configured for transporting eggs from the receptacle to the imaging system. The conveyor system may comprise a rotatable disc having, along its periphery, a plurality of openings for receiving the eggs.

The conveyor system may further comprise a first holding element and a second holding element configured to guide the eggs through the imaging system. The holding elements may comprise a flexible and transparent strip of sheet material having a substantially rectangular shape. The sheet may be made from regenerated cellulose, e.g.

CELLOPHANE (TM), but other flexible and transparent materials may be used. The conveyor system may comprise a first storage reel holding a storage of the first holding element and second storage reel holding a storage of the second holding element.

The conveyor system may comprise a transfer device which is configured to transfer eggs from the disc to the first holding element. The transfer device may comprise a picking element which is configured to pick the eggs from the openings and position them onto the first holding element between the first and second holding elements. The picking element may comprise a suction tube having a distal end which is configured, by means of a negative pressure in the suction tube, to pick an egg from the opening of the disc.

The pressing device may comprise a pressing head configured to press on the holding elements to compress and flatten eggs deposited therebetween by the transfer device.

Above-discussed preferred and/or optional features of each aspect of the invention may be used, alone or in appropriate combination, in the other aspects of the invention.

Following drawings are appended to facilitate the understanding of the disclosure and the invention as it is defined in the claims:

5 Fig. 1 is a schematic illustration of a fish egg analysis system according to one embodiment of the invention;

Fig. 2 is a schematic illustration of a fertilized egg from a teleost fish species at an early development stage;

10 Fig. 3 is a flow chart schematically illustrating a method according to one embodiment of the invention;

Fig. 4 schematically illustrates an embodiment of an imaging system.

Fig. 5 is a schematic illustration of a fish egg analysis system according to a further embodiment of the invention;

15 Fig. 6 is a flow chart schematically illustrating a method according to one embodiment of the invention;

Fig. 7 illustrates a fish egg analysis apparatus according to an embodiment of the invention;

Fig. 8 illustrates the fish egg analysis apparatus according to Fig. 7 in an alternative perspective view; and

Fig. 9 shows a detailed part-view of the apparatus according to Fig. 7.

20 In the drawings, like reference numerals have been used to indicate common parts, elements or features unless otherwise explicitly stated or implicitly understood by the context.

In the following, one or more specific embodiments of the invention will be described in more detail with reference to the drawings. However, it is specifically intended that the invention is not limited to the embodiments and illustrations contained herein but includes
25 modified forms of the embodiments including portions of the embodiments and combinations of elements of different embodiments as come within the scope of the following claims. It should be appreciated that in the development of any such actual implementation, as in any engineering or design project, numerous implementation- specific decisions must be made to achieve the developer's specific goals, such as compliance with
30 system and/or business-related constraints, which may vary from one implementation of the invention to another. Moreover, it should be appreciated that such a development effort might be complex and time consuming, but would nevertheless be a routine undertaking of design, fabrication and manufacture for the skilled person having the benefit of this disclosure.

Fig. 1 illustrates, schematically, a fish egg analysis system 100 for detecting fertilized fish eggs and determining the fertilization rate of a batch of fertilized fish eggs. The system 100 comprise a fixation system 102 where a sample of eggs 104, drawn from the batch of mixed fish spermatozoa and eggs, is mixed with a fixative 106. The system 100 further comprises an imaging system 108 where images of the fixed eggs 110 are taken and an image analysis system 112 where the images are analysed. The system 100 also comprises a conveyor system 114 configured to pass the fixed eggs past the imaging system 108 so that one or a plurality of images can be taken of each egg.

The fixation system 102 may comprise a fixation station where the sample 104 and the fixative 106 are mixed manually. Alternatively, the fixation system 102 may comprise an automated fixations station where the fixative 106 is added to the sample 104 in an automated fashion.

In the shown embodiment, the conveyor system 114 comprises a funnel 116 configured for receiving the fixed eggs 110 and a tubular conduit 118 arranged at the bottom of the funnel 116. The conveyor system 114 also comprises a receptacle 120 into which the conduit 118 opens. Water is arranged to flow from the funnel 116, through the conduit 118 and to the receptacle 120, transporting the eggs through the system. A pump (not shown) may be used to pump water from the receptacle 120 back to the funnel 116. The eggs, however, are separated from the water and discarded once having arrived in the receptacle 120.

The tubular conduit 118 has an inner diameter D which is within the range of $D_{\text{egg}} < D < 2 \cdot D_{\text{egg}}$, where D_{egg} is the diameter of the eggs the system 100 is to analyse. In other words, the diameter of the conduit 118 is larger than the diameter of the eggs the system 100 is to analyse but smaller than two times this diameter, thus allowing the fixed eggs 110 to be brought through the conduit 118 in single file (also, see Fig. 4 which shows the conduit 118 in a cross-sectional view). For example, if the eggs to be analysed are Salmon eggs (*Salmo salar*), the inner diameter D of the conduit may be approximately 10 mm. It is understood that the system 100 may be configured in a modular fashion allowing for easy substitution of the conduit 118 to a conduit adopted to the species of eggs to be analysed.

The conduit 118 may be made from transparent plastic or glass.

The imaging system 108 is positioned adjacent the conduit 118 and comprises a camera 122 configured to take images of individual eggs as they pass. The camera 122 is connected to the image analysis system 112 allowing the images to be transferred to the same for analysis.

A method of determining a fertilization rate of a batch of mixed fish spermatozoa and eggs using the system 100 will now be discussed in more detail.

According to the method, fertilization is established by analysing and detecting cell division, or cleavage, in the eggs. In fish eggs, cleavage occurs only in a blastodisc, i.e. a thin region of yolk-free cytoplasm at an animal pole of the egg. Most of the rest of the egg cell is full of yolk. Fig. 2 schematically illustrates a fertilized egg 200 from a teleost fish species at an

early development stage. The egg 200 comprises a blastodisc 202 having undergone initial cell cleavage to form a blastomere. In the illustration, the blastomere 202 has four cells. The blastomere 202 is positioned at an animal pole 204 of the egg, close to an outer membrane 206 of the egg.

- 5 The method comprises the initial step 302 (see Fig. 3) of drawing a sample comprising a plurality of eggs from the batch of mixed fish spermatozoa and eggs.

In a next step 304, the drawn sample is treated with a fixative bringing the outer membrane 206 of the eggs of the sample at least partly transparent and/or the blastodisc of the eggs at least partly opaque. Such fixatives are known in the art and may include acetic acid-alcohol-formaldehyde solutions, such as Serra's fixative. Other known fixtures are Stockard's
10 solution and AquaBoost® Quattro. However, any fixative capable of bringing the eggs transparent enough and/or the blastodisc visible enough so that cell development can be seen can be used.

The fixative will inhibit further cell development of the eggs and, consequently, the sample
15 should not be treated with the fixative too early. Fertilized eggs must be allowed sufficient time to develop in order to enable them to be distinguished from un-fertilized eggs. The sample may be drawn from the batch and treated with the fixative at approximately 48 hours from initial mixing of the spermatozoa and eggs. During this period the blastodisc 202 of the fertilized eggs will typically have developed 2 to 16 cells forming a blastomere.

20 Generally, depending on the fish species and environmental factors, such as the temperature of the incubator, the sample may typically be drawn and treated with the fixative within a time period from initial mixing of the fish spermatozoa and the eggs within the range of 30 to 220 degree-hours. Eggs of the species *S. salar* may advantageously be treated with the fixative within the range of 170 to 190 degree-hours, e.g. at 180 degree-hours.

25 In a next step 306, the treated sample is entered into the conveyor system 114 (see Fig. 1) and the eggs are brought to pass the imaging system 108 in single file allowing the camera 122 to take one or a plurality of images of each egg. Since the blastodisc 202 is located at the animal pole 204 of the egg 200 (see Fig. 2), the system 100 may have, instead of a single imaging system 108, a plurality of imaging systems 108' positioned at different locations
30 along the conduit 118, wherein each system 108' comprises a camera 122'. This will allow the eggs time to rotate between the systems 108', thus increasing the likelihood that at least one of the cameras 122' captures an image of the blastodisc 202. In such a configuration, it may be advantageous if the two imaging systems 108' are positioned on either side of a bend in the conduit 118, thereby further increasing the chances that the cameras 122' capture
35 different sides of the egg.

In a further configuration, shown in Fig. 4, one or a plurality of mirrors or prisms 124 may be used to capture images of the egg from different directions using the same camera 122". In such an imaging system 108", a plurality of images of the egg from different directions

can be captured simultaneously in a single exposure. In principle, such a configuration will allow the camera 122" to simultaneously capture images of the egg 200 from four orthogonal directions. If the configuration is employed in two imaging systems position on either side of a bend of the conduit 118, e.g. in the systems 108' in Fig. 1, it can almost be guaranteed
 5 that at least one of the captured images contains a clear view of the blastodisc 202 even if the egg, due to inertia, maintains its orientation in space throughout its travel through the conduit 118.

In a next step 308, the images of each egg are transferred to the image analysis system 112, 112' where the images are analysed for signs of normal cell division, i.e. cell division
 10 corresponding to normal development of the egg. If such cell division is detected in any of the images, the egg is classified as fertilized. If no cell division is detected, the egg is classified as un-fertilized.

The analysis may employ computer vision and algorithms built by machine learning systems utilising mathematical models based on sample or training images of eggs known to be
 15 fertilized.

Fig. 5 schematically illustrates another embodiment of a fish egg analysis system 400 for detecting fertilized fish eggs and determining the fertilization rate of a batch of fertilized fish eggs.

The system 400 comprise a fixation system 402 where a sample of eggs 404, drawn from
 20 the batch of mixed fish spermatozoa and eggs, is mixed with a fixative 406. The fixation system 402 may be the same as described in Fig. 1, it may comprise a fixation station where the sample 404 and the fixative 406 are mixed manually or, alternatively, the fixation system 402 may comprise an automated fixations station where the fixative 406 is added to the sample 404 in an automated fashion.

The system 400 further comprises an imaging system 408 where images of the fixed eggs
 25 410 are taken and an image analysis system 412 where the images are analysed.

The system 400 also comprises a conveyor system 414 configured to pass the fixed eggs past the imaging system 408 so that one or a plurality of images can be taken of each egg. In the shown embodiment, the conveyor system 414 comprises a first holding element 416 and a
 30 second holding element 418 configured to trap the fixed eggs between them. Each holding element 416, 418 may comprise a longitudinal strip of a flexible sheet material and at least one of the holding elements 416 may be made from a transparent material. The holding elements 416, 418 may for example be made from sheets of regenerated cellulose, e.g. CELLOPHANE (TM).

The system 400 also comprises a pressing device 420 configured to press onto the holding
 35 elements 416, 418 to compress and flatten eggs trapped therebetween. The pressing device 420 may comprise a pressing head 424 arranged on one side of the holding elements acting against support element 428 arranged on the other side of the holding elements. The pressing

device 420 operates on the holding elements 416, 418 and an egg trapped therebetween to compress the egg. The compressive action should preferably be sufficiently large to allow the outer membrane of the egg to rupture but sufficiently small not to damage the blastomere, i.e. the blastodisc under cleavage. This will allow cytoplasm to escape the outer cell membrane but leave the blastomere inside the flattened egg.

It is understood that the thickness to which the egg should preferably be compressed may vary between egg species. For eggs of the species *S. salar*, the predefined thickness may preferably be within the range of 0.1 to 1.5 mm; more preferably within the range of 0.3 to 1.0 mm; and even more preferably within the range of 0.4 to 0.6 mm, e.g. 0.5 mm.

In the pressing device 420, the holding elements 416, 418 becomes orientated parallel to each other with the flattened egg housing the blastomere trapped between them.

After the pressing device 420, the flattened egg is transported between the holding elements 416, 418 to the imaging system 408. The imaging system 408 is positioned adjacent the holding elements 416, 418 and comprises a camera 422 oriented with its focal plane trained between the parallel holding elements 416 and 418, i.e. coinciding with the flattened egg. The camera 422 is configured to take at least one image of each flattened egg as the eggs are guided through the imaging system 408, thus also capturing images of the blastodiscs/blastomeres of the eggs.

The imaging system 408 may comprise an illumination device 427 arranged on a side of the holding elements 416, 418 being opposite the camera 422. In such a configuration, it is advantageous that both holding elements 416 and 418 are made from a transparent material, thus allowing transmission light microscopy techniques to be used for capturing the image. However, other illumination techniques may be used.

The camera 422 is connected to the image analysis system 412 allowing the images to be transferred to the same for analysis.

A method of determining a fertilization rate of a batch of mixed fish spermatozoa and eggs using the system 400 will now be discussed in more detail with reference to Fig. 6.

The method comprises the initial step 502 of drawing a sample comprising a plurality of eggs from the batch of mixed fish spermatozoa and eggs. In a next step 504, the drawn sample is treated with a fixative bringing the outer membrane 206 of the eggs (see Fig. 2) of the sample at least partly transparent and/or the blastodisc of the eggs at least partly opaque. As previously discussed, such fixatives are known in the art and may include acetic acid-alcohol-formaldehyde solutions, such as Serra's fixative. Other known fixtures are Stockard's solution and AquaBoost® Quattro. However, as previously stated, any fixative capable of bringing the eggs transparent enough and/or the blastodisc/blastomere visible enough so that cell development can be seen can be used.

As also previously discussed, the fixative will inhibit further cell development of the eggs and, consequently, the sample should not be treated with the fixative too early. Fertilized eggs must be allowed sufficient time to develop in order to enable them to be distinguished from un-fertilized eggs. The sample may be drawn from the batch and treated with the
 5 fixative at approximately 48 hours from initial mixing of the spermatozoa and eggs. During this period the blastodisc 202 of the fertilized eggs will typically have developed 2 to 16 cells. As in the method previously discussed with reference to Fig. 3, the sample may typically be drawn and treated with the fixative within a time period from initial mixing of the fish spermatozoa and the eggs within the range of 30 to 220 degree-hours. Eggs of the
 10 species *S. salar* may advantageously be treated with the fixative within the range of 170 to 190 degree-hours, e.g. at 180 degree-hours. Eggs of other species may, depending on the fish species and environmental factors, such as the temperature of the incubator, be treated with the fixative within other ranges.

In a next step 504, the treated sample is entered into the conveyor system 414 (see Fig. 5)
 15 and in a subsequent step 505, the eggs are compressed and flattened in the pressing device 420 (see Fig. 5), thereby allowing cytoplasm to escape the outer cell membrane but leaving the blastomere inside the flattened egg, as previously discussed.

The fixative may affect the viscosity of cytoplasm (make it more viscous). Therefore, in order to press cytoplasm out of the egg in a controlled manner, it may be advantageous to
 20 effectuate the pressing step within a predefined time period from said step of treating the drawn sample with the fixative. For eggs of the species *S. salar*, it may be advantageously to effectuate the pressing step no later than 2 to 3 hours from treating the drawn sample with the fixative.

In a next step 506, the eggs are brought to pass the imaging system 408 in single file allowing
 25 the camera 422 to take one or a plurality of images of each egg. Since the blastodisc 202 is orientated substantially in a plane parallel to the holding elements 416, 418 (as a consequence of the egg having been compressed and flattened in the pressing device 420), the blastodisc/blastomere 202 will be positioned in the focal plane of the camera, thus allowing a clear image of the blastodisc/blastomere 202 to be captured.

In a next step 508, the images of each egg are transferred to the image analysis system 412
 30 where the images are analysed for signs of normal cell division, i.e. cell division corresponding to normal development of the egg. If such cell division is detected in an image, the egg associated with that image is classified as fertilized. If no cell division is detected in the image, the egg is classified as un-fertilized.

The analysis may employ computer vision and algorithms built by machine learning systems
 35 utilising mathematical models based on sample or training images of eggs known to be fertilized.

Figs. 7-9 show an embodiment of a fish egg analysis apparatus 600 which may be used for determining the fertilization rate of a batch of fertilized fish eggs.

The apparatus 600 comprises a receptacle 628 including an inlet 629 for receiving a sample of fish eggs, e.g. taken from a batch of fertilized fish eggs. In the present embodiment, the
 5 receptacle 628 has the form of a substantially rectangular open-top tray.

The apparatus 600 also comprise an imaging system 608 including an image-capturing device in the form of a camera 622 and an image analysis system. The image analysis system is housed inside the apparatus 600 and, therefore, is not visible in the figures.

The apparatus 600 further comprises a conveyor system 614 configured to transport eggs
 10 from the receptacle 628 to the imaging system 608. The conveyor system 614 comprises a rotatable disc 630 having, along its periphery, a plurality of openings 632. The disc 630 is rotatably arranged about a substantially horizontal axis extending above and across the receptacle 628. The disc 630 has a diameter which is sufficient to allow the disc 630 to extend down into the receptacle 608 and allow eggs fed to the receptacle to be captured by
 15 the openings 632 when they are in a lower-most position during rotation of the disc (see Fig. 9).

The receptacle 628 may comprise one or a plurality of guide groves 634 configured for guiding the eggs towards and into the openings and a spillway passage 636 allowing fluid in which the eggs are transported into the receptacle 628 to be separated from the eggs and
 20 conducted to an outlet 637 and ejected. The disc 630 may be recessed in a side-wall of the receptacle 628, e.g. as is shown in Fig. 9.

For eggs of the species *S. salar*, experiments have shown that the openings 632 may have a diameter of approximately 6.5 mm and the disc may have a thickness of approximately 3 mm to allow effective capture of the eggs. Generally, however, it is understood that the
 25 dimensions of the openings 632 should be adapted to the species of eggs to be analysed.

The conveyor system 614 further comprises a first holding element 616 and a second holding element 618 configured to guide the eggs through the imaging system 608. In the present embodiment, both holding elements 616, 618 comprise a flexible and transparent strip of sheet material having a substantially rectangular shape. The sheet may be made from
 30 regenerated cellulose, e.g. CELLOPHANE (TM), but other flexible and transparent materials may be used. The conveyor system 614 comprises a first storage reel 638 holding a storage of the first holding element 616 and second storage reel 640 holding a storage of the second holding element 618.

The conveyor system 614 comprises a substantially rectangular support element 626
 35 extending from above the receptacle 628 and through the imaging system 608. The support element 626 displays a substantially horizontal, upward-facing support surface 642 on which the first holding element 616, running from the first storage reel 638, and eventually also the second holding element 618, running from the second storage reel 640, are supported. The

support element 626 extends substantially parallel to the disc 630 with the support surface 642 extending substantially orthogonal to the rotational plane of the disc at a level between the apex and the centre of the disc.

5 A guide roll 644 (see Fig. 8) forms a nip with the support surface 642 through which the holding elements 616, 618 are guided. A pair of drive rolls 646, 648, arranged at a downstream end of the support element 626, are arranged to advance the holding elements 616, 618 through the imaging system 608.

10 The conveyor system 614 further comprises a transfer device 650 which is configured to transfer eggs, raised by the disc 630, from the disc 630 to the first holding element 616 (see Figs. 7 and 9). The transfer device 650 comprises a picking element 652 (see Fig. 9) which is configured to pick the eggs from the openings 632 and position them onto the first holding element 616 upstream of the guide roll 644 and between the first and second holding elements 616, 618, as can be seen most clearly in Fig. 7. In the present embodiment, the picking element comprises a pair of suction tubes which are movable in the axial direction
15 of the disc 630 by means of a pneumatically operated cylinder-piston arrangement 654.

The system 600 also comprises a pressing device 620 configured to compress and flatten the eggs prior to them entering the imaging system 608 (see Fig. 8). The pressing device 620 is arranged between the storage reels 638, 640 and the guide roll 644 and comprises a pressing head 624 arranged above the first holding element 616. The pressing head 624 is movable
20 in a vertical direction by means of a pneumatically operated cylinder-piston arrangement 656 and is arranged to interact with the support device 626 at the position of the transfer device 650 to press onto the holding elements 616, 618 to compress and flatten the eggs deposited therebetween by the transfer device 650.

25 In operation, a suspension comprising eggs to be analysed is entered into the receptacle 628 through the inlet 629. In the receptacle 628, eggs are captured by the openings 632 in the rotating disc 630 when they are in a lower-most position. The fluid in which the eggs are transported into the receptacle 628 is conducted to the outlet 637 and ejected (see Fig. 9).

30 The rotating disc 630 raises the captured eggs above the support element 626 and the suction tubes 652 of the transfer device 650 are extended towards the disc 630 by the cylinder-piston arrangement 654. A distal end of each suction tube 652 is brought into contact with an egg positioned in an opening (see also Fig. 7). A negative pressure in the suction tube 652 attaches the egg to the distal end and the suction tube is then retracted to position the distal end above the first holding element 616. The negative pressure in the suction tube is then released and the egg deposited onto the first holding element 616. As can most clearly be
35 seen in Fig. 7, the transfer device 650 positions the eggs onto the first holding element 616 upstream of the guide roll 646 and between the first and second holding devices 616, 618.

When the transfer device 650 has positioned the eggs onto the first holding element 616, the pressing head 624 of the pressing device 620 is lowered and the egg or eggs trapped between

the holding devices 616, 618 are compressed and flattened to a predefined thickness. As previously discussed, for eggs of the species *S. salar*, the predefined thickness may preferably be within the range of 0.1 to 1.5 mm; more preferably within the range of 0.3 to 1.0 mm; and even more preferably within the range of 0.4 to 0.6 mm, e.g. 0.5 mm.

- 5 The first and second holding devices 616, 618 may be heat-sealed to each other during the pressing operation, joining the holding devices 616 and 618 to each other along a path that continuously or discontinuously encircles the flattened egg.

When the pressing device 620 has compressed and flattened the eggs (and possibly also heat-sealed the holding devices 616, 618), the drive rolls 646, 648 advance the holding devices
 10 616, 618 and the flattened egg trapped therebetween to the guide roll 644 and, subsequently, to the imaging system 608. The guide roll 644 may preferably provide a nip with the support surface 642 that corresponds to the pressing thickness of the pressing device 620. In the imaging system, the camera 622 takes one or a plurality of images of each flattened egg. The imaging system 608 may comprise an illumination device 627 arranged below the first
 15 holding device 616, allowing light to be transmitted from the illumination device 627 through the flattened egg and into the camera 622. The illumination device 627 may advantageously be recessed in the support surface 624.

The images captured by the camera 622 are transferred to the image analysis system to be analysed. The image analysis system may comprise a central processing unit (CPU), volatile
 20 and/or non-volatile memory and data input and output devices. In the disclosed embodiment, the apparatus comprises an output device 658 in the form of a computer screen.

If the apparatus is used to determine the fertilization rate of the analysed eggs, the image analysis system analyses the images for signs of normal cell division, i.e. cell division corresponding to normal development of the egg. If such cell division is detected in an
 25 image, the egg associated with that image is classified as fertilized. If no cell division is detected in the image, the egg is classified as un-fertilized. As previously described, this analysis may employ computer vision and algorithms built by machine learning systems utilising mathematical models based on sample or training images of eggs known to be fertilized.

- 30 The eggs entering the receptacle 628 may be fixed eggs, i.e. eggs that have been subjected to a fixation operation as previously disclosed. However, for species of eggs where the blastodisc is visible without having been fixed, such an operation may be dispensed with.

It is appreciated that certain features of the invention, which, for clarity, have been described above in the context of separate embodiments, may also be provided in combination in a
 35 single embodiment. Conversely, various features of the invention, which, for brevity, have been described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. In particular, it will be appreciated that features described in

relation to one particular embodiment may be interchangeable with features described in relation to the other embodiments.

In the preceding description, various aspects of the apparatus according to the invention have been described with reference to the illustrative embodiment. For purposes of explanation,
5 specific numbers, systems and configurations were set forth in order to provide a thorough understanding of the apparatus and its workings. However, this description is not intended to be construed in a limiting sense. Various modifications and variations of the illustrative embodiment, as well as other embodiments of the apparatus, which are apparent to person skilled in the art to which the disclosed subject-matter pertains, may lie within the scope of
10 the present invention as defined by the following claims.

Claims

1. A method of identifying fertilized fish eggs (200) in a sample of eggs (104) taken from a batch of incubating fish eggs, the method comprising the steps of:
 - 5 - within a period of time from initial fertilization of the batch, treating the sample (104) with a fixative (106) bringing an outer membrane (206) of the eggs at least partly transparent and/or a blastodisc (202) of the eggs at least partly opaque;
 - capturing at least one image of individual eggs of the sample depicting the blastodisc (202) of the egg;
 - 10 - analysing the at least one image for detecting divided cells in the blastodisc (202) of the egg; and
 - identifying each egg associated with an image showing divided cells in the blastodisc (202) as a fertilized egg.
2. The method according to claim 1, wherein said step of capturing at least on image of individual eggs (200) comprises capturing a plurality of images of each egg from a plurality of directions.
3. The method according to claim 1, wherein said step of capturing at least one image of individual eggs (200) comprises capturing, in a single exposure, a plurality of images of each egg from a plurality of directions.
- 20 4. The method according to claim 3, characterised by using one or a plurality of prisms and/or mirrors (124) to capture, in said single exposure, said images of the egg (200) from said plurality of directions.
5. The method according to any one of the preceding claims, wherein said step of capturing at least one image of individual eggs (200) comprises bringing the eggs, one-
25 by-one, passed an imaging system (108, 108', 108").
6. The method according to any one of the preceding claims, wherein said period of time from initial fertilization of the batch to treating the sample (104) with the fixative (106) is within the range of 30 to 220 degree-hours.
7. The method according to any one of the preceding claims, wherein said fixative
30 comprises any one of: an acetic acid-alcohol-formaldehyde solution (Serra's fixative) Stockards solution, or AquaBoost® Quattro.
8. The method according to claim 1, **characterised by** the step of compressing the egg to a predetermined thickness prior to said step of capturing said at least one image of the egg.

9. The method according to claim 8, wherein the predefined thickness is any one of:
within the range of 0.1 to 1.5 mm; within the range of 0.3 to 1.0 mm; within the range
of 0.4 to 0.6 mm; and 0.5 mm.
10. The method according to any one of claims 8 and 9, wherein said step of compressing
the egg comprises the sub-steps of:
 - trapping the egg between two holding elements (616, 618); and
 - bringing the holding elements (616, 618) together compressing the trapped egg.
11. The method according to claim 10, wherein said holding elements (616, 618)
comprises ductile strips of transparent sheet material.
12. The method according to claim 11, wherein said transparent sheet material comprises
regenerated cellulose.
13. The method according to any one of claims 8-12, wherein said step of capturing an
image of the compressed egg comprises the sub-steps off:
 - arranging an image-capturing device (422, 622) on a first side of the compressed
egg;
 - arranging a light source (427, 627) on a second side of the compressed egg, said
second side being opposite the first side; and
 - transmitting light from the light source (427, 627) through the compressed egg and
into the image-capturing device (422, 622).
14. A fish egg analysis system (100, 400) **characterised by**:
 - at least one imaging system (108, 108', 108'', 408) being configured for capturing at
least one image of individual fish eggs of a sample (104) of eggs comprising
fertilized (200) and unfertilized fish eggs depicting the blastodisc (202) of the eggs;
 - an image analysis system (112, 112', 412) being configured for analysing the
captured images for detecting divided cells in the blastodisc (202) and identifying
each egg associated with an image showing divided cells in the blastodisc (202) as
a fertilized egg; and
 - a conveyor system (114, 414) arranged for conveying the eggs of the sample past
the at least one imaging system (108, 108', 108'', 408) in single file.
15. The system (100) according to claim 14, **characterised by** a fixation system (102)
configured for mixing a sample (104) of eggs comprising fertilized (200) and
unfertilized fish eggs with a fixative (106) bringing an outer membrane (206) of the
eggs at least partly transparent and/or a blastodisc (202) of the eggs at least partly
opaque.
16. The system (100) according to any one of claims 14 and 15, **characterised in that** said
at least one imaging system (108, 108', 108'') comprises a first imaging system (108')

and a second imaging system (108') being positioned one after the other along conveyor system (114).

17. The system (100) according to any one of claims 15 and 16, **characterised in that** the conveyor system (114) comprises a tubular conduit (118) for conveying the eggs, the conduit (118) having an inner diameter D within the range of $D_{\text{egg}} < D < 2 \cdot D_{\text{egg}}$, where D_{egg} is the diameter of the eggs of the sample.
18. The system (100) according to any one of claims 14-18, **characterised in that** said at least one imaging system (108, 108', 108'') is configured for capturing images of individual eggs (200) from a plurality of directions.
19. The system (100) according claim 18, **characterised in that** each of said at least one imaging system (108, 108', 108'') comprises one or a plurality of mirrors or prism allowing a camera (122, 122', 122'') of the imaging system (108, 108', 108'') to capture, in a single exposure, a plurality of images of each egg from different directions.
20. The system (400) according to any one of claims 14 and 15, **characterised by** a pressing device (420) arranged upstream of said at least one imaging system (408), said pressing device (420) being configured to compress each egg to a predetermined thickness.
21. The system (100, 400) according to any one of claims 14-19, **characterised in that** said image analysis system (112, 112') employs an algorithm built by a machine learning system utilising mathematical models based on sample or training images of eggs known to be fertilized.
22. A fish egg analysis apparatus (600) comprising:
 - an imaging system (408) configured for capturing images of fish eggs;
 - an image analysis system (412) configured for analysing the images captured by the imaging system (408); and
 - a conveyor system (414) arranged for guiding the eggs through the imaging system (408) allowing the imaging system (408) to capture at least one image of each individual egg,**characterised by** a pressing device (420) configured to compress each fish egg to a predetermined thickness prior to the conveyor system (414) guiding the egg through the imaging system (412).

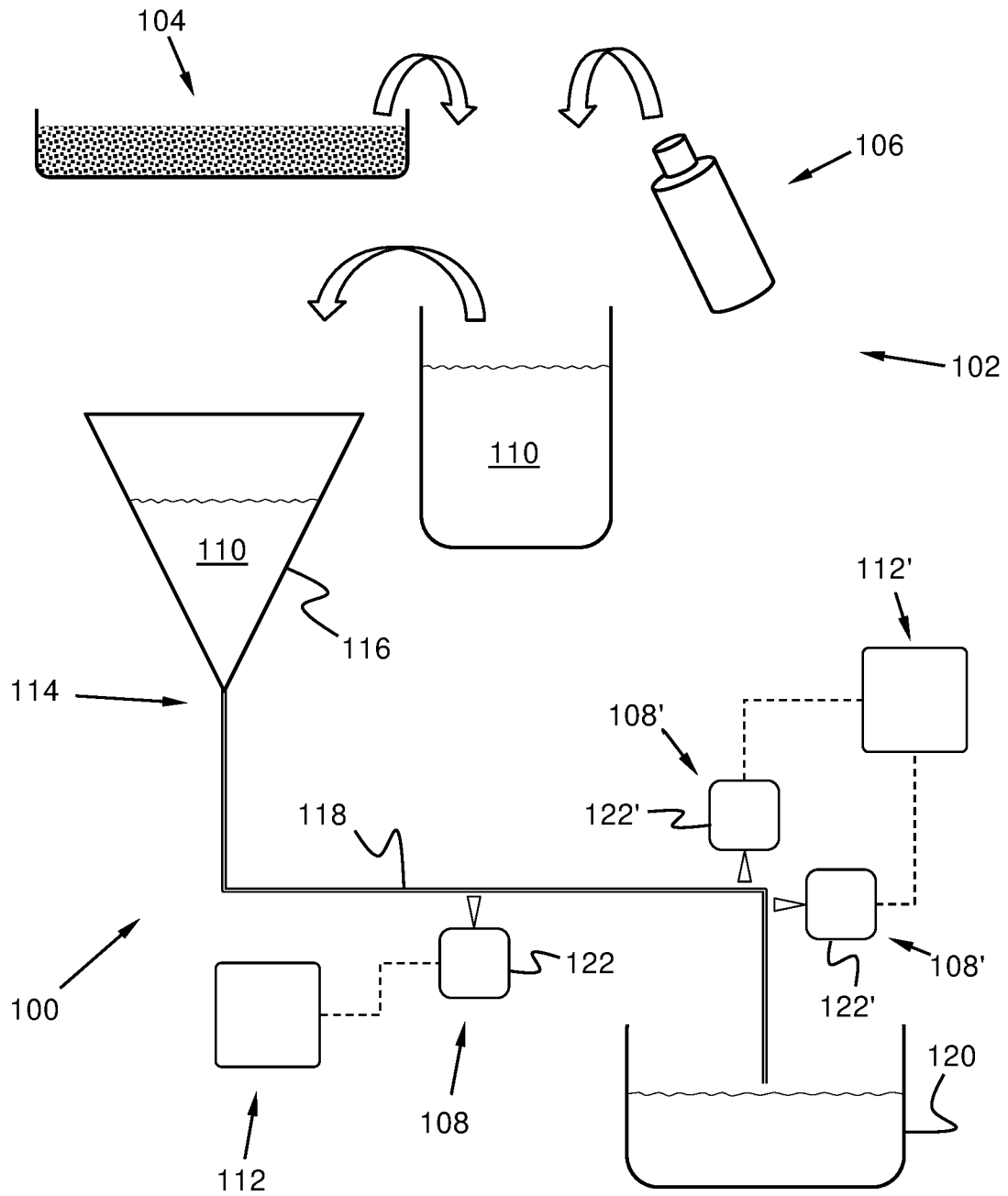


Fig. 1

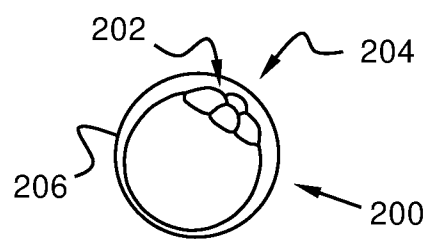


Fig. 2

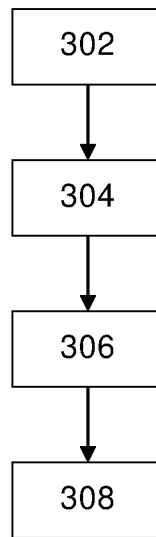


Fig. 3

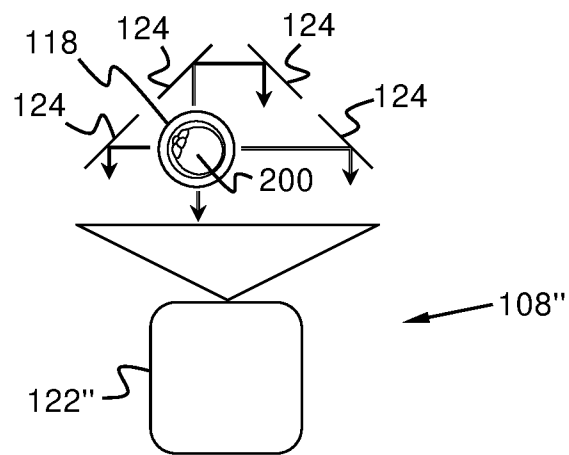
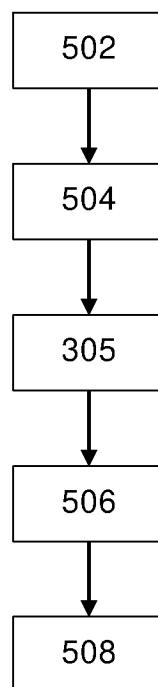
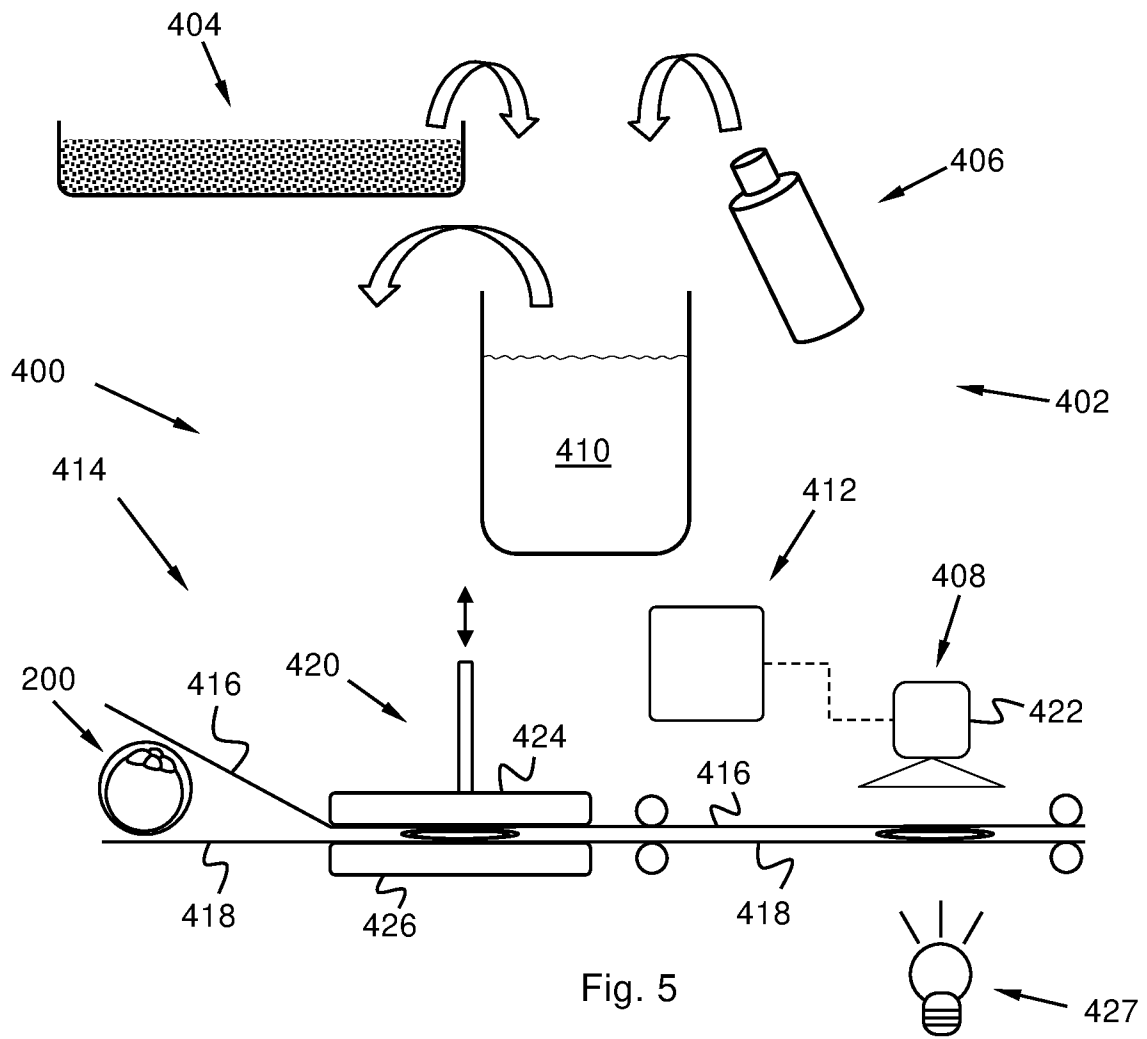


Fig. 4



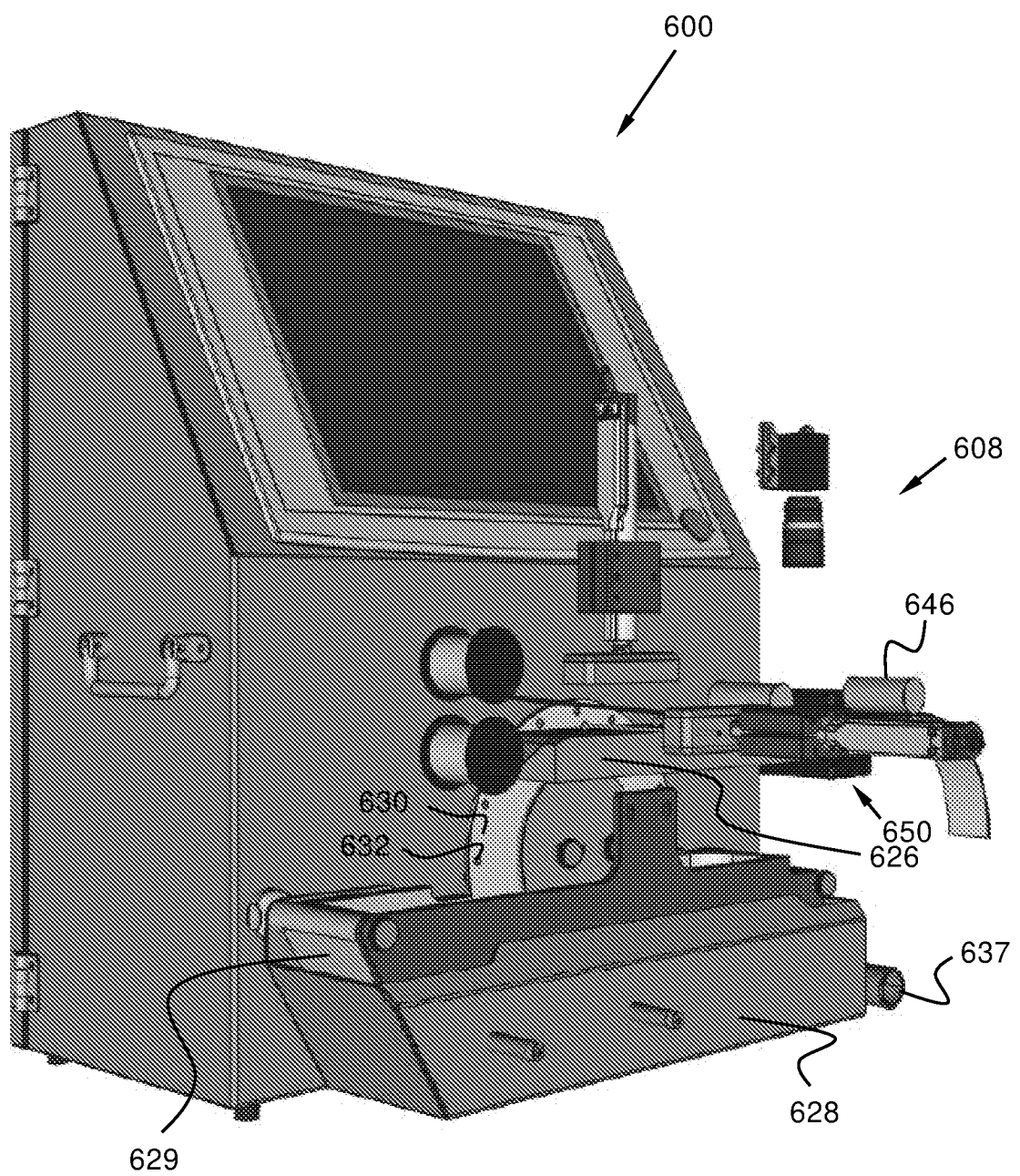


Fig. 7

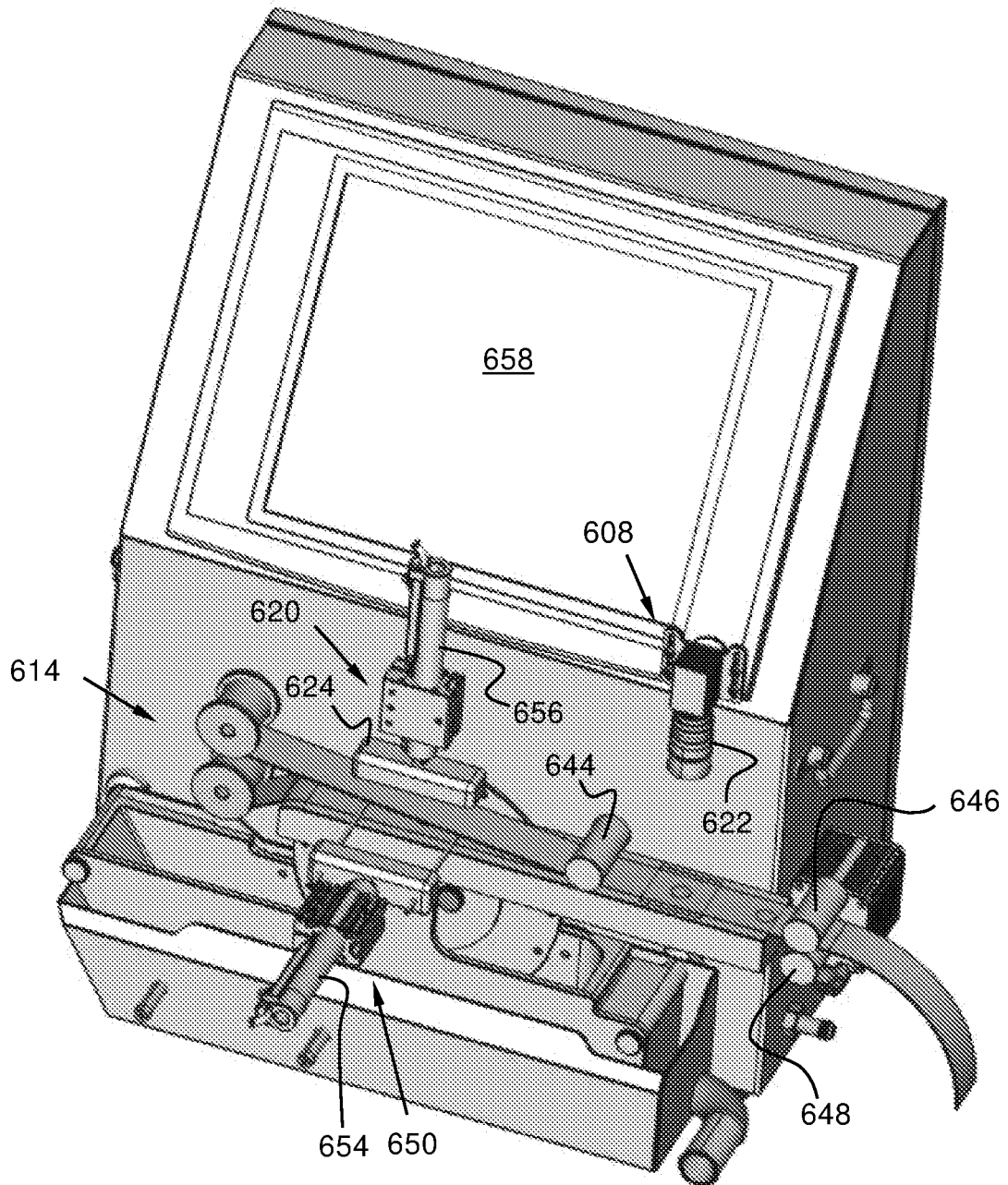


Fig. 8

Fig. 9