

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 June 2009 (25.06.2009)

PCT

(10) International Publication Number
WO 2009/079474 A1

(51) International Patent Classification:
C12N 15/89 (2006.01) *C12M 1/26* (2006.01)

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(21) International Application Number:
PCT/US2008/086871

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(22) International Filing Date:
15 December 2008 (15.12.2008)

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA,
CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE,
EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID,
IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK,
LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW,
MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT,
RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ,
TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM,
ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/007,701 14 December 2007 (14.12.2007) US

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(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL,

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[Continued on next page]

(54) Title: METHODS, SYSTEMS, AND COMPUTER READABLE MEDIA FOR FACILITATING AUTOMATION OF BLAS-
TOCYST MICROINJECTION

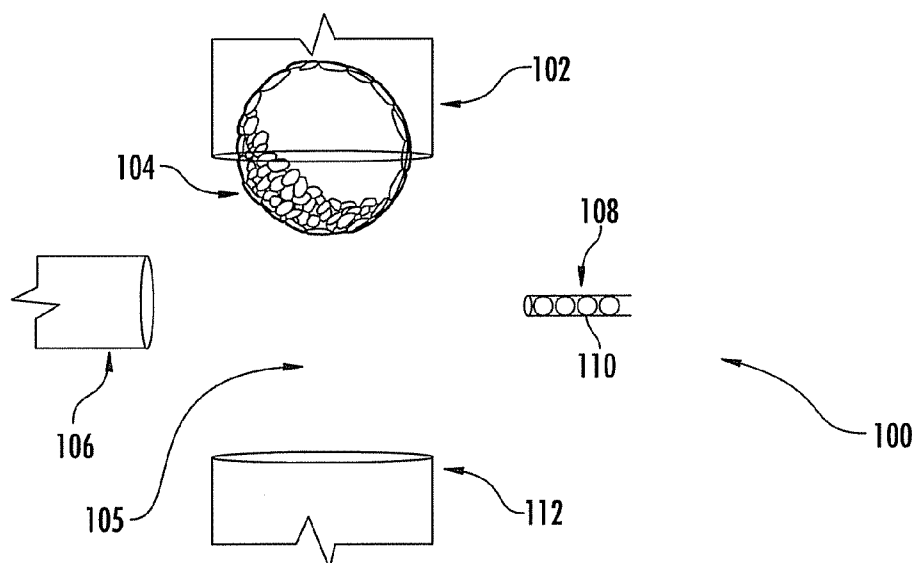


FIG. 1

(57) Abstract: Methods, systems, and computer readable media for facilitating automation of blastocyst microinjection are disclosed. According to one method, a target blastocyst is automatically fed, from among a plurality of blastocysts, into a blastocyst microinjection area. The target blastocyst is automatically held in a fixed position within the blastocyst microinjection area and oriented so as to be suitable for injection. One or more cells are injected into the target blastocyst. After injection of the one or more cells, the target blastocyst is automatically released and removed from the blastocyst microinjection area.

WO 2009/079474 A1



NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG,
CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— *before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments*

Published:

— *with international search report*

METHODS, SYSTEMS, AND COMPUTER READABLE MEDIA FOR FACILITATING AUTOMATION OF BLASTOCYST MICROINJECTION

This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/007,701 filed December 14, 2007; the disclosure of which is incorporated herein by reference in its entirety.

The subject matter described herein relates to blastocyst microinjection. More specifically, the subject matter relates to methods, systems, and computer readable media for facilitating automation of blastocyst microinjection.

Microinjection of materials into target cells, such as blastocysts, has conventionally been a manual process. In conventional microinjection, trained experts place a target cell into an injection area that is viewed with the aid of a microscope. The target cell is then oriented and held in place, typically using a holding pipette exerting suction on the target cell, while the operator injects other cells, such as embryonic stem (ES) cells, through appropriate portion of the cellular wall. This is accomplished using a small needle (e.g., injection pipette) containing the material to be injected capable of penetrating the target cell wall. The injection pipette is manually controlled in the x, y, and z directions using a manual control, such as a joystick. After injection, the target cell is removed from the injection area, placed back in the pool of target cells, and a next target cell is obtained.

Conventional microinjection is prone to human error. For example, inserting the injection pipette into the cell with too much force may damage the cell making it unsuitable for further use. Inserting the injection pipette into an incorrect portion of the cell wall may also result in damage to the cell (e.g., improper orientation of the cell).

Additionally, conventional microinjection is a slow and tedious process resulting in an expensive process. For example, manually orienting a cell, locating a candidate cell from among a pool of cells, and manipulating the injection pipette are slow procedures.

- 5 These problems have conventionally been addressed by providing extensive training for human operators in order to build a network of experienced operators. While such operators generally do perform higher quality injections in less time than inexperienced operators, the cost of training operators is high.
- 10 Accordingly, in light of these difficulties, a need exists for improved methods, systems, and computer readable media for facilitating automation of blastocyst microinjection.

SUMMARY

- 15 Methods, systems, and computer readable media for facilitating automation of blastocyst microinjection are disclosed. According to one method, a target blastocyst is automatically fed, from among a plurality of blastocysts, into a blastocyst microinjection area. The target blastocyst is automatically held in a fixed position within the blastocyst microinjection area
- 20 and oriented so as to be suitable for injection. One or more cells are injected into the target blastocyst. After injection of the one or more cells, the target blastocyst is automatically released and removed from the blastocyst microinjection area.

- A system for facilitating automation of blastocyst microinjection is also
- 25 disclosed. The system includes a feeder pipette for automatically feeding a target blastocyst from among a plurality of blastocysts into blastocyst microinjection area. A holding pipette automatically holds the target blastocyst in a fixed position within the blastocyst microinjection area and releases the target blastocyst from the blastocyst microinjection area after
- 30 injection of the one or more cells. An injection pipette orients the target blastocyst so as to be suitable for injection and injects one or more cells into the target blastocyst. A collection pipette automatically removes the target

blastocyst from the blastocyst microinjection area after injection of the one or more cells.

A system-on-a-chip for facilitating automation of blastocyst microinjection is also disclosed. The system includes an integrated circuit
5 having a plurality of channels forming a fluidic communication path for blastocysts and at least one blastocyst microinjection area in the fluidic communications path. The integrated circuit forms a suction hole in the blastocyst microinjection area for holding the blastocyst in place. At least one electrode is configured to electrorotate the blastocyst so as to be
10 suitable for injection. A microelectromechanical (MEMS) needle injects one or more cells into the target blastocyst. A sensor observes the position and orientation of the injection area.

The subject matter described herein for facilitating automation of blastocyst microinjection may be implemented using a computer program
15 product comprising computer executable instructions embodied in a tangible computer readable medium that are executed by a computer processor. Exemplary computer readable media suitable for implementing the subject matter described herein includes disk memory devices, programmable logic devices, and application specific integrated circuits. In one implementation,
20 the computer readable medium may include a memory accessible by a processor. The memory may include instructions executable by the processor for implementing any of the methods for routing a call described herein. In addition, a computer readable medium that implements the subject matter described herein may be distributed across multiple physical
25 devices and/or computing platforms.

BRIEF DESCRIPTION OF THE DRAWINGS

The subject matter described herein will now be explained with reference to the accompanying drawings of which:

30 Figure 1 is a diagram of an exemplary four-pipette system for facilitating automation of consecutive blastocyst microinjection according to an embodiment of the subject matter described herein;

Figure 2A is a diagram showing exemplary steps for performing an automated microinjection task according to an embodiment of the subject matter described herein;

5 Figure 2B is a flow chart of an exemplary process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

Figure 3 is a flow chart of an exemplary blastocyst delivery process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

10 Figure 4 is a flow chart of an exemplary blastocyst capture process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

Figure 5 is a flow chart of an exemplary blastocyst release process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

Figure 6 is a flow chart of an exemplary blastocyst orientation process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

20 Figure 7 is a flow chart of an exemplary blastocyst collection process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

Figure 8 is a flow chart of an exemplary process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

25 Figure 9 is an exemplary image data showing a blastocyst microinjection area before and after image processing for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

Figure 10 is a diagram of an exemplary control system for providing pipette motion control for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

Figure 11 is a flow chart of an exemplary embryonic stem cell delivery process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

5 Figure 12 is a diagram showing an exemplary system for counting of the number of delivered embryonic stem cells for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

10 Figure 13 is a block diagram of an exemplary system for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

Figures 14A and 14B are diagram of an exemplary physical arrangement of a system for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein;

15 Figure 15A is an overhead view of a system-on-a-chip system for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein; and

20 Figure 15B is cross-sectional view of a system-on-a-chip system for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein.

DETAILED DESCRIPTION

Figure 1 is a diagram of an exemplary system for facilitating automation of consecutive blastocyst microinjection that includes four
25 pipettes. Conventionally, blastocyst microinjection has been accomplished using a two-pipette system wherein a holding pipette is used to obtain a target blastocyst from a collection of possible blastocysts, held in place while an injection pipette is used to inject cells (e.g., embryonic stem cells) into the blastocyst. Upon completion of injection, the blastocyst is returned to the
30 collection of blastocysts and a second blastocyst may be retrieved. As described above, this process is labor intensive, slow, and prone to error.

System **100** shows a four-pipette system which adds two pipettes (i.e., a feeder pipette and a collection pipette) to conventional holder pipette

and injection pipette systems. Pipettes included within system 100 may be associated with micropositioners, computer-controlled micrometer syringes, and other related components (not shown) for facilitating automation of consecutive blastocyst microinjection. Referring to system **100**, feeder

5 pipette **102** may obtain a single blastocyst **104** from a collection of blastocysts and deliver blastocyst **104** to injection area **105**. For example, in order to accommodate typical sizes of blastocyst **104**, the diameter of feeder pipette **102** may be approximately 120um to allow for the entry of a single blastocyst without distortion. Upon entering injection area **105**, blastocyst

10 **104** may be temporarily fixed to holding pipette **106** via suction. Next, blastocyst **104** may be oriented so as to be suitable for injection. One exemplary technique may include the push-pull technique for rotating blastocyst **104** so as to orient a portion of the cell wall of blastocyst **104** opposite holding pipette **106**. Injection pipette **108** may then be inserted into

15 blastocyst **104** and one or more cells **110** may be injected into blastocyst **106**. Finally, holding pipette **106** may release blastocyst **104**, and collection pipette may remove blastocyst **104** from injection area **105**.

The addition of feeder pipette **102** and collection pipette **112** to microinjection system **100** simplifies the design and implementation of

20 control algorithms for automation. Simplification occurs because feeding and collecting blastocysts directly to and from the injection area avoids the need to move the XY stage to search for them. Consequently, system **100** reduces the number of control variables in the system by reducing the number of moving parts during microinjections.

25 Additional pipettes **102** and **112** also simplify the requirements for video processing algorithms by keeping the injected and the non-injected blastocysts separated from each other. Therefore, vision algorithms are not required to differentiate between multiple blastocyst states (e.g., pre-injection and post-injection). In addition, system **100** may be capable of processing

30 multiple consecutive blastocyst microinjections (i.e. a production line) without user intervention. Such a configuration may reduce the amount of time required for microinjections, thus contributing to improved productivity. Additional inputs for user control of feeder and collection micrometer

syringes may, for example, be implemented using keys of a computer keyboard for providing additional user control in semi-automated embodiments.

5 In addition to the advantages described above, four-pipette system **100** described above has the potential to speedup multiple blastocyst microinjections by avoiding the need to search for the embryos (i.e., the working well) since they are kept at known locations and by avoiding the need to identify non-injected blastocysts because injected and non-injected blastocysts are kept separated from each other.

10 System **100** was evaluated by conducting microinjection experiments under teleoperated manual control. The performed experiments showed that system **100** may be appropriate for automated blastocyst microinjections. First, it was found that applying suction to the holding pipette prior to the delivery of a blastocyst, the blastocyst was captured immediately once it was
15 fed to the injection area, i.e., as soon as it exits the feeder pipette, a similar result was observed for blastocyst collection, i.e., the creation of suction on the collection pipette prior to the blastocyst's release from the holder causes it to move directly from one pipette to the other once it is released.

In comparative studies, the performance of a semi-automated
20 (joystick teleoperated) embodiment of microinjection system **100** was tested against a traditional manual microinjection system. There was a dramatic reduction in operator training time and there was an associated increase in transgenic mice Chimera birth rates. Microinjection success rates, commonly between 40%-70% were greater than 80% for both the
25 experienced expert and the novice using this new system. Similarly, the performance of a fully-automated embodiment of microinjection system **100** was tested against a traditional manual microinjection system where a high-level control system automatically penetrated and extracted the injection pipette during blastocyst microinjection. The automated system was 75%
30 successful in the injection process and the injected blastocysts were implanted and developed to term. The automated microinjection system gave a 53.3% birth rate and yielded 20% Chimeras.

Figure 2A is a diagram showing exemplary steps for performing an automated microinjection task according to an embodiment of the subject matter described herein. Referring to Figure 2A, an automate microinjection task may begin at block **200** wherein a blastocyst is delivered to the injection area. In block **202**, the blastocyst may be captured (i.e., held in place by holding pipette **106**) and oriented in block **204**. Next, injection process **206** may be composed of several steps. For example, in blocks **208** and **210**, an injection needle may approach and penetrate the target blastocyst, respectively. This process may be aided by using image analysis techniques that enhance or highlight the boundaries of the injection needle and the blastocyst cell wall, as well as control techniques for accurately controlling the movement of the injection needle. This is illustrated in block **210** as pipette motion control, which will be described in greater detail below. After penetration of the blastocyst, cells may be injected into its interior in block **214**. The injection needle may then be retracted in step **216**, with the help of pipette motion control **210**. Finally, the blastocyst may be released in step **218**, and collected with other blastocysts which have been previously injected in step **220**.

Figure 2B is a flow chart of an exemplary process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. The steps shown in Figure 2B may refer to software routines executable by a processor of a computer. Referring to Figure 2B, beginning at step **222**, an auto-inject command may initiate the automatic injection of consecutive blastocysts. This may be aided, as shown in step **224**, by pre-loading a feeder pipette with multiple blastocysts such that the system may repeat the injection process for each blastocyst without user intervention. Initially, the injection pipette may be moved to a "home" position corresponding to a known, pre-determined location relative to the injection area as well as other components such that distances may be easily calculated for performing accurate pipette motion control.

Beginning at step **226**, a procedural loop may begin by delivering a next blastocyst from among a plurality of blastocysts queued in the feeder pipette to the injection area. In step **228**, the blastocyst may be captured by

the holding pipette. In step **230**, the blastocyst may be oriented if necessary using one or a variety of possible techniques. The blastocyst is then injected in step **230** and collected (i.e., removed from the injection area and placed in a collection area) in step **234**. While the process shown in Figure 2B may be
5 fully automated, in the event of errors, it may be advantageous to provide an opportunity between blastocyst injections to allow for user input and/or correction. Thus, step **236** allows for the user to continue and repeat the automated injection process for the next blastocyst or allow them to exit the process and perform some action. Accordingly, if the user continues the
10 process, control returns to step **226** and alternatively, may end at step **238**.

Figure 3 is a flow chart of an exemplary blastocyst delivery process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. Referring to Figure 3, at step **300**, a feed blastocyst command may feed a single blastocyst into the
15 injection area. For example, multiple blastocysts may be queued in feeder pipette and dispensed into the injection area individually. The delivery of a blastocyst to the injection area may be automated by commanding the feeder micrometer syringe to slowly rotate clockwise until a blastocyst was delivered and positively identified in the injection area. In step **302**, updated
20 image data of the blastocyst is obtained. For example, the position and orientation of the blastocyst may be determined by detecting the boundaries of the blastocyst relative to the known boundaries of the XY stage using an optical sensor, such as a microscope and associated CCD camera. In step **304**, it is determined whether the blastocyst is present in the image. If the
25 blastocyst is not present in the image, then a command is issued in step **306** to perform a clockwise step on the feeder micrometer syringe. Next, no action is performed a short amount of time in order to allow for the fluid response in step **308**, after which control returns to step **302** where updated blastocyst information is obtained from the vision system (step **302**). Now, if
30 the blastocyst is present in the image (step **304**), then control proceeds to step **310** where the process is completed.

Figure 4 is a flow chart of an exemplary blastocyst capture process for facilitating automation of blastocyst microinjection according to an

embodiment of the subject matter described herein. Referring to Figure 4, beginning at step **400**, a capture blastocyst command is issued in order to hold the blastocyst in a fixed position using suction applied by the holding pipette. In other words, as soon as a new blastocyst enters the injection area it should be captured by the holding pipette. Similar to the process described above in Figure 3, in step **402**, updated image data of the blastocyst is obtained. For example, the position and orientation of the blastocyst may be determined by detecting the boundaries of the blastocyst relative to the known boundaries of the XY stage or other objects within the injection area (e.g., the holding pipette). In step **404**, it is determined whether the blastocyst is next to the tip of the holding pipette. If the blastocyst is not next to the tip of the holding pipette, then a command is issued in step **406** to perform a clockwise step on the holder micrometer syringe. Next, no action is taken for a short amount of time in order to allow for the fluid response in step **408**, after which control returns to step **402** where updated blastocyst information is obtained from the vision system. Now, if the blastocyst is next to the tip of the holding pipette (step **404**), then control proceeds to step **410** where the process is completed.

In one example, the algorithm shown in Figure 4 assumes that a blastocyst is present in or near the injection area, and commands the holder micrometer syringe to rotate counterclockwise until the blastocyst is captured by the holding pipette. The task is deemed completed when the blastocyst is located next to the holding pipette tip, i.e., when the following equations are true:

$$(X_{blast} - R_{blast}) < X_{holder}$$

Equation 1

$$Y_{holder} - 0.25R_{blast} < Y_{blast} < Y_{holder} + 0.25R_{blast}$$

Equation 2

In the equations above, (X_{holder}, Y_{holder}) are the coordinates of the holding pipette tip, (X_{blast}, Y_{blast}) are the coordinates of the blastocyst center,

and R_{blast} is the blastocyst radius. All of these parameters were obtained in real-time from the vision processing system.

Figure 5 is a flow chart of an exemplary blastocyst release process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. There are basically two situations in which the blastocyst must be released from the holding pipette during orientation adjustments prior to injection and during its collection after microinjection. As may be appreciated from Equations 3-5 below, these situations may basically correspond to the inverse of the Equations 1 and 2 above for blastocyst capture. During blastocyst release, the holder micrometer syringe may be slowly rotated clockwise until the blastocyst is no longer next to the holding pipette tip. Exemplary decision equations for task completion may be given by:

$$(X_{blast} - R_{blast}) > X_{holder}$$

Equation 3

$$Y_{blast} > Y_{holder} + 0.25R_{blast}$$

Equation 4

$$Y_{blast} < Y_{holder} - 0.25R_{blast}$$

$$(X_{blast} - R_{blast}) < X_{holder}$$

Equation 5

Thus, a blastocyst release task may be deemed completed when any one of Equations 3-5 is true. Referring to Figure 5, beginning at step **500**, a release blastocyst command is issued in order to release the blastocyst from the holding pipette. In step **502**, updated image data of the blastocyst is obtained. For example, the position and orientation of the blastocyst may be determined by detecting the boundaries of the blastocyst relative to the known boundaries of the XY stage or other objects within the injection area (e.g., the holding pipette). In step **504**, it is determined whether the blastocyst is next to the tip of the holding pipette. If the blastocyst is not next to the tip of the holding pipette, then a command is issued in step **506** to perform a clockwise step on the holder micrometer syringe. Next, no action is taken for a short amount of time in order to allow for the fluid response in

step **508**, after which control returns to step **502** where updated blastocyst information is obtained from the vision system. Now, if the blastocyst is next to the tip of the holding pipette (step **504**), then control proceeds to step **510** where the process is completed.

5 Figure 6 is a flow chart of an exemplary blastocyst orientation process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. During preparation for injection, the blastocyst's orientation must be verified and adjusted. This is necessary to avoid injecting through the ICM. Here, the push-pull method of
10 blastocyst orientation may be used for automation. This method was selected for its simplicity. As a result, the algorithm for automatic blastocyst orientation consisted of releasing and capturing the blastocyst until an acceptable orientation for injection was detected. Referring to Figure 6, beginning at step **600**, an adjust blastocyst orientation command is issued in
15 order to adjust the orientation of the blastocyst. In step **602**, updated image data of the blastocyst is obtained. For example, the position and orientation of the blastocyst may be determined by detecting the boundaries of the blastocyst relative to the known boundaries of the XY stage or other objects within the injection area (e.g., the holding pipette). In step **604**, it is
20 determined whether the orientation of the blastocyst is acceptable for injection. If the orientation of the blastocyst is not acceptable for injection, then commands are issued in steps **606** and **608**, respectively, to release and re-capture the blastocyst. Next, control returns to step **602** where updated blastocyst orientation information is obtained from the vision
25 system. If the blastocyst is the orientation of the blastocyst is acceptable for injection, then control proceeds to step **610** where the process is completed.

 Figure 7 is a flow chart of an exemplary blastocyst collection process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. Blastocyst collection is
30 performed after the injection pipette retracts from the blastocyst. This task completes the microinjection cycle, so it involves releasing the blastocyst from the holding pipette and collecting it through suction on the collection pipette. Referring to Figure 7, beginning at step **700**, a collect blastocyst

command is issued in order to remove the blastocyst from the injection area and place it in a collection area. Ideally, this collection area serves to separate injected blastocysts from un-injected blastocyst in order to save time in identifying them. In step **702**, a command is issued to perform a
5 clockwise step on the collector micrometer syringe. A command is then issued in step **704** to release the blastocyst, where it is automatically removed from the injection area. In step **706**, it is determined whether the blastocyst is present in the image of the injection area. If the blastocyst is not present in the image, then a command is issued in step **708** to perform a
10 clockwise step on the holder micrometer syringe. Next, no action is taken for a short amount of time in order to allow for the fluid response in step **710**, after which control returns to step **704** where another blastocyst release command is issued. If the blastocyst is no longer present in the injection area, then control proceeds to step **712** where the process is completed.

15 Figure 8 is a flow chart of an exemplary process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. Referring to Figure 8, in step **800**, the process of injecting a blastocyst may be initiated. Initially, the holding and injection pipettes may be aligned (horizontally) in step **802**. The injection
20 pipette may then be moved to the edge of the blastocyst's outer membrane in step **804**, after which the piezo injector may be turned on in step **806**. Next, in step **808**, the injection pipette may be moved to the center of the blastocyst by piercing the outer membrane. The piezo injection may then be turned on in step **810**, and one or more cells (e.g., embryonic stem cells)
25 may be delivered into the blastocyst in step **812**. Next, the injection pipette may be retracted from the blastocyst in step **814** and moved to its home position in step **816**. The injection process is thus completed in step **818**, and may be repeated automatically for a next target blastocyst.

Figure 9 shows exemplary image data of a blastocyst microinjection
30 area before and after image processing for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. Referring to Figure 9, original image **900** includes shows unprocessed image data acquired from a microscope coupled to a digital

camera and displayed on a computer display. Image **900** shows objects located within the blastocyst microinjection area including holding pipette **902**, target blastocyst **904**, and injection pipette **906**. Image **900** represents what is conventionally seen by operators trying to manually inject
5 blastocysts. It is appreciated that while somewhat useful, a lack of contrast or other highlighting of areas of interest within the image for facilitating injection typically results in slower injections that are more prone to error.

In contrast, processed image **908** shows the result of image **900** after image analysis is performed. Holding pipette **902**, target blastocyst **904**, and
10 injection pipette **906** may all be more readily seen because the contrast ratio has been increased so that objects may appear brighter and whiter than the background, which appears darker. Additionally, bounding boxes **904** and **906** may be added to image **908** to highlight the boundaries of holding pipette **902** and injection pipette **906**, respectively. By adding bounding
15 boxes **904** and **906**, either a human operator or a computer-controlled micropositioner may more accurately determine the position of holding pipette **902** and injection pipette **906** and therefore may more accurately, quickly, and consistently inject target blastocyst **904**.

Figure 10 is a diagram of an exemplary control system for providing
20 pipette motion control for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. One of the main system characteristics that may have a major impact on the success of blastocyst microinjections is the quality of the injection pipette motion control. Motion control for manipulating a micromanipulation robot
25 may be based on visual servoing techniques. In one embodiment, all motion commands may be computed solely based on visual feedback, i.e., on localization information provided by a video processing system. In such an embodiment, robot motions cause changes to captured video images, which result in changes to the vision system's output parameters. An exemplary
30 control structure may consist of a PID (proportional-integral-derivative) control loop in which position errors are computed directly from 2-D image parameter space. For example, target coordinates and injection pipette tip coordinates may be defined with respect to an image reference frame.

Position errors used for control may be obtained from the difference between those two points, as defined by Equation 6.

$$\bar{E} = \bar{T} - \bar{P}$$

5 **Equation 6**

where $\bar{T} \in \mathbb{R}^2$ is the target location, and $\bar{P} \in \mathbb{R}^2$ is the pipette location, both defined in terms of image coordinates;

$$\bar{T} = \begin{bmatrix} X \ t \\ Y \ t \end{bmatrix}$$

Equation 7

10
$$\bar{P} = \begin{bmatrix} X \ p \\ y \ p \end{bmatrix}$$

Equation 8

Referring to Figure 10, a relationship between system variables is shown, including control parameters: proportional gain (Kp), integral gain (Ki) and derivative gain (Kd). These parameters may be used for control system calibration and provide the appropriate mapping of commands generated in an image space to motion commands in a target space.

PID control equations for an exemplary 2-D visual servoing system may be given by:

20
$$\bar{E}i(k) = \bar{E}i(k-1) + \frac{\Delta t}{2} (\bar{E}(k) + \bar{E}(k-1))$$

Eq. 8.9

$$\bar{C}(k) = K p \bar{E}(k) + K i \bar{E}i(k) + K d \frac{(\bar{E}(k) - \bar{E}(k-1))}{\Delta t}$$

Eq. 8.10

25 where $\bar{E}i$ is the integral of the error signal; Δt is the sample period; and

$\bar{C} = \begin{bmatrix} C_x & C_y \end{bmatrix}^T$ is the motion command vector sent to the robot driver.

The control system configuration shown in Figure 10 restricts the task space to 2-D during the microinjection operations. This restriction means that the robot was constrained to move along the X and Y axes only, guaranteeing that the vertical alignment of the holding and injection pipettes was not affected. Furthermore, the control system may be configured to compensate for small misalignments between the robot and the image axes in order to successfully avoid expensive system calibration.

Thus, the movement of the injection pipette may be based on the visual servoing system described above (i.e., injection pipette insertion, delivery of ES cells, and pipette retraction). The algorithm shows that, once a target coordinate is commanded, the visual servoing system takes the pipette to the desired location. In one embodiment, this positioning process is stopped, and the task is deemed completed, when the error between the target the actual pipette position is small (less than 2 pixels).

Figure 11 is a flow chart of an exemplary embryonic stem cell delivery process for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. The delivery of ES cells inside the blastocyst cavity was not automated in this research. Consequently, we required an operator to manually perform this task using the joystick. Referring to Figure 11, beginning at step **1100**, a deliver embryonic stem cells command is issued in order to inject one or more ES cells into the blastocyst. In step **1102**, a command is issued to the vision system to begin counting the number of cells delivered. The number of delivered cells are totaled in step **1104** and compared to a predetermined desired number in step **1106**. If it is determined that not enough ES cells have been delivered, then a command is issued in step **1108** to perform a clockwise step on the holder micrometer syringe. Next, no action is taken for a short amount of time in order to allow for the fluid response in step **1110**, after which control returns to step **1104** where an updated count of the number of delivered cells is obtained. If enough ES cells have been delivered, then control proceeds to step **1112** where the process is completed.

The automated of this part of the system is deemed future work. Getting this task automated requires the development of extra video processing algorithms to detect and count the number of delivered ES cells. This is not a trivial task because the ES cells are very small and typically
5 move very fast inside the injection pipette.

Figure 12 is a diagram showing an exemplary system for counting of the number of delivered embryonic stem cells for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. A small section of the injection pipette may be monitored
10 for the detection of cells passing through it. This detection may be possible by defining a small monitoring area which can only detect one cell at a time. In this way, the ES cells count can be incremented every time a new cell is detected in the defined area. For example, injection pipette **1200** may be inserted into target blastocyst **1202** and contain one or more ES cells **1204**.
15 Monitoring area **1206** may be defined at an area approximately equal to the size of one of cells **1204** and may be located at the intersection of injection pipette **1200** and the outer membrane of blastocyst **1202**. As ES cells **1204** pass through monitoring area **1206**, they may be counted using a vision system. This location for monitoring area **1206** is likely to be an ideal
20 detection site because it avoids background image noise from blastocyst inner structures, and because the image focus in that part of the pipette is close to the focus on the pipette's tip.

Figure 13 is a block diagram of an exemplary system for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. Referring to Figure 13, system **1310** may
25 include blastocyst injection area **1302**. For example, a Petri dish containing an area suitable for maintaining blastocysts during the microinjection process may be located on an anti-vibration table in order to increase the accuracy of each injection. Blastocyst dispensing pipette **1304** may be located near injection area **1302** so that blastocyst may be delivered to
30 injection area **1304**. When a blastocyst enters injection area **1302**, holding pipette **1306** may hold the blastocyst in place. Injection pipette **1308** may injection one or more cells into the blastocyst and collection pipette **1310**

may remove the blastocyst from injection area **1302** after injection. Thus, the exemplary configuration shown in system **1300** is based on four micromanipulation robots (i.e., micromanipulators **1314**, **1318**, **1322**, and **1332**) and four motorized micrometer syringes (i.e., micrometer syringes **1316**, **1320**, **1330**, and **1334**).

In the configuration shown in Figure 13, injection area **1302** may be magnified by microscope **1312** in order view objects and actions occurring inside of injection area **1302**.

Dispensing pipette **1104** may be controlled by microcontroller **1314** and blastocysts may be fed into dispensing pipette **1304** using motorized micrometer syringe **1316**. There is unlikely to be a need for manual control of dispensing pipette **1104**, and thus, the embodiment shown in system **1300** shows a fully automated control of dispensing pipette **1304**.

In contrast, holding pipette **1306** may be controlled by manual micromanipulator **1318**, and may be fed by a motorized micrometer syringe **1320**. This is because, as described above, it may be advantageous in some situations to allow user control of holding pipette **1306**. However, ideally, fully automated control of holding pipette **1306** may be maintained.

Injection pipette **1308** may be associated with piezo microinjector **1322** for injecting one or more cells into target blastocysts. Piezo microinjection **1322** may be controlled by micromanipulation robot **1324**. Micromanipulation robot **1324** may further be associated with robot driver **1328** for sending commands to robot **1324** in order to physically manipulate its position and/or orientation. Robot driver **1328** may receive commands from motion control software located on a desktop computer for automatically manipulating robot **1324**. Alternatively, robot **1324** may be controlled by manual micromanipulator **1326** for semi-automated embodiments of system **1300**. Finally, motorized micrometer syringe **1330** may be associated with injection pipette **1308** for injecting one or more cells into target blastocysts. It is appreciated that micrometer syringe **1330** may also be operated using commands issued by motion control software located on a desktop computer.

After injection of one or more cells, the target blastocyst may be collected and removed from injection area **1302** using collection pipette **1310**. Associated with collection pipette **1310** may be micropositioner **1332** and motorized micrometer syringe **1334**. Similar to motorized micrometer syringe **1330**, motorized micrometer syringe **1334** may be controlled using motion control software located on a desktop computer.

Blastocyst injection area **1302** may, for example, be located within a Petri dish or other suitable environment. In the embodiment shown in system **1300**, injection area **1302** may be physically located on top of XY stage **1338**. XY stage **1338** may include a flat platform made of glass having a rigid frame (e.g., metal) so as to provide a platform for viewing blastocysts for injection. XY stage **1338** may be manipulated by XY stage driver **1336**, such as a DC servo motor and rotary encoder, for providing accurate positioning, flatness, straightness, orthogonality, and repeatability.

Additionally, in order to perform digital image analysis on images of injection area **1302**, charge coupled device (CCD) sensor-based camera **1340** may be associated with microscope **1312**. CCD camera **1340** may obtain images of injection area **1302** and send the digital images to a desktop computer for analysis. For example, desktop computer **1342** may contain one or more software modules, stored in memory and executable by a processor, for receiving images of injection area **1302**, modifying the images for distinguishing key features of blastocysts etc., displaying the images, and receiving user input for controlling micromanipulators **1314**, **1318**, **1322**, and **1332**.

For example, digital images may be received by frame grabber **1342** from CCD camera **1340** and sent to image processing module **1346**. Image processing module **1346** may output images to a display, such as monitor **1348** associated with computer **1342**. Additionally, the captured images may be forwarded to an experiment data recorder **1350** for analyzing data extracted from the images such as the number of blastocysts injected, the number of cells injected in each blastocyst, the number of errors, etc. Also associated with experiment data recorder **1350** may be a motion control module **1352** for controlling the movement of micromanipulators **1314**, **1318**,

1322, and **1332**. Motion control module may include instructions executable by computer **1342** for implementing one or more of the algorithms described above.

In one embodiment, a conventional inverted microscope (having multiple objectives) may be replaced by a microscope **1312** having a single objective and a motorized set of precision zoom lenses that can be directly attached to video camera **1340**. Additional light necessary for illuminating injection area **1302** may be provided by a fiber optic illuminator (not shown). Finally, user commands manager module **1354** may be associated with motion control module **1352** for managing input (e.g., commands) received from the user. For example, user commands module **1354** may be connected to a keyboard **1356** and a joystick **1358** for manually manipulating micromanipulators **1318** and **1326**.

Figures 14A and 14B are diagram of an exemplary physical arrangement of a system for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. Referring to the view shown in Figure 12A, XY stage **1338** may support a Petri dish or other suitable container for implementing a blastocyst injection area. An inverted microscope **1312** may be located above XY stage **1338** and associated with CCD camera **1340** for obtaining image of the injection area. Motorized micrometer syringes **1316**, **1320**, **1330**, and **1334** are located in proximity to XY stage **1338** in order to, for example, dispense blastocysts into the injection area, hold and orient the blastocysts for injection, and remove the blastocyst from the injection area after injection. Manual micromanipulators **1318** and **1326** may be located in a position suitable for use by a user for controlling injection pipette **1308** and holding pipette **1306**. Piezo injection **1322** may be associated with injection pipette **1306** for injecting one or more cells into a target blastocyst. It is further appreciated that, as described above, piezo injection **1322** may be connected to motion control module **1352** located separately on desktop computer **1342**.

Referring to Figure 14B, Petri dish **1400** may be located centrally on top of XY stage **1338**. Surrounding Petri dish **1400** may be feeder pipette

1304, holding pipette **1306**, injection pipette **1308**, and collection pipette **1310**.

Figure 15A is an overhead view of a system-on-a-chip system for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. Referring to Figure 15A, system-on-a-chip **1500** may include integrated circuit **1502** having four channels **1504**, **1506**, **1508**, and **1510**. The injection area may be located at the intersection of channels **1504**, **1506**, **1508**, and **1510**. Each of channels **1504-1510** may be filled with a fluid suitable for sustaining the viability of the blastocyst during injection. Because the size of channels **1504-1510** is small (e.g., channel diameters of around 100 nanometers to several hundred micrometers), microfluidic properties dominate the behavior of the fluid in the channel. The behavior of fluids at the microscale can differ from macrofluidic behavior in that factors such as surface tension, energy dissipation, and fluidic resistance start to dominate. For example, in microfluidic conditions, the Reynolds number, which characterizes the presence of turbulent flow, is extremely low, and thus the flow will remain laminar (i.e., two fluids joining will not mix readily via turbulence, so diffusion alone must cause the two fluids to mingle). In Figure 15A, blastocyst **1512** may enter the injection area via channel **1504**. Once inside in the injection area, blastocyst **1512** may be held in place using micro-suction hole **1510** located beneath the injection area. Blastocyst **1512** may then be oriented using electrorotation applied by electrode **1516**. Electrorotation (ER) is the circular movement of an electrically polarized particle, such as blastocyst **1512** through manipulation of an applied electric field. Once properly oriented, MEMS needle **1518** may be used to inject one or more cells into blastocyst **1512**. For example, MEMS needle **1518** may be a silicon tube having a diameter so as to enable only one cell to be injected at a time.

Figure 15B is cross-sectional view of a system-on-a-chip system for facilitating automation of blastocyst microinjection according to an embodiment of the subject matter described herein. Referring to Figure 15B, blastocyst **1512** may enter the injection area via feeder channel **1304** (i.e., the left). Micro-suction hole **1514** may be located centrally within the injection

area and directly below blastocyst **1512**. Optical sensor **1318** may be located above blastocyst **1512** for capturing image data of the injection area. It is appreciated that optical sensor **1518** replaces microscope **1312** in other embodiments, and may therefore be easier, cheaper, and more accurate. It

5 is also appreciated that while an optical sensor is shown in system **1500**, other sensors may also be used without departing from the scope of the subject matter described herein. In order to illuminate blastocyst **1512**, a light source may be located opposite optical sensor **1518**, such as light emitting diode (LED) **1520**. Electrodes **1522** may be used to orient

10 blastocyst **1512** using 3-D electrorotation. MEMS needle **1518** may be inserted into blastocyst **1512** in order to inject one or more cells. Finally, blastocyst **1512** may be removed from the injection area via collection channel **1506** (i.e., the right) using fluid flow within the channel. During the injection process, blastocyst **1512** may be cooled using the Peltier effect.

15 One advantage of the system-on-a-chip embodiment shown in Figures 15A and 15B includes eliminating the need for a microscope and a micromanipulator.

It will be understood that various details of the subject matter described herein may be changed without departing from the scope of the

20 subject matter described herein. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation, as the subject matter described herein is defined by the claims as set forth hereinafter.

CLAIMS

What is claimed is:

1. A method for facilitating automation of blastocyst microinjection, the method comprising:
 - 5 automatically feeding a target blastocyst from among a plurality of blastocysts into a blastocyst microinjection area;
 - automatically holding the target blastocyst in a fixed position within the blastocyst microinjection area;
 - orienting the target blastocyst so as to be suitable for injection;
 - 10 injecting one or more cells into the target blastocyst; and
 - automatically releasing and removing the target blastocyst from the blastocyst microinjection area after injection of the one or more cells.
2. The method of claim 1 wherein orienting the target blastocyst includes
15 using a push-pull technique.
3. The method of claim 1 wherein orienting the target blastocyst includes semi-automatically orienting the target blastocyst using a graphical user interface (GUI) and a user input device to vary the orientation of the target blastocyst.
- 20 4. The method of claim 1 wherein orienting the target blastocyst includes automatically orienting the target blastocyst using image analysis.
5. The method of claim 1 wherein injecting one or more cells into the target blastocyst includes injecting one or more embryonic stem cells.
6. A system for facilitating automation of blastocyst microinjection, the
25 system comprising:
 - a feeder pipette for automatically feeding a target blastocyst from among a plurality of blastocysts into blastocyst microinjection area;
 - a holding pipette for automatically holding the target blastocyst
30 in a fixed position within the blastocyst microinjection area and releasing the target blastocyst from the blastocyst microinjection area after injection of the one or more cells;

an injection pipette for orienting the target blastocyst so as to be suitable for injection and injecting one or more cells into the target blastocyst; and

- 5 a collection pipette for automatically removing the target blastocyst from the blastocyst microinjection area after injection of the one or more cells.
7. The system of claim 6 comprising a magnifier for enlarging an image of the injection area.
8. The system of claim 6 wherein the magnifier includes one of a single
10 objective microscope, a multi-objective microscope, a fixed lens microscope, and a zooming lens microscope.
9. The system of claim 6 comprising a camera for recording images of the injection area and converting the images to digital image data.
10. The system of claim 6 comprising one or more micropositioners for
15 manipulating the position of at least one of the feeder pipette and the dispensing pipette.
11. The system of claim 6 comprising one or more micromanipulators for manipulating the position of at least one of the holding pipette and the injection pipette.
- 20 12. The system of claim 11 wherein the one or more micromanipulators are manually controllable by a human operator.
13. The system of claim 6 comprising a pipette control module for manipulating at least one of the holding pipette and the injection pipette.
- 25 14. The system of claim 6 comprising one or more syringes associated with at least one of the feeder pipette, the holding pipette, the injection pipette, and the collection pipette.
15. The system of claim 6 comprising a syringe control module for manipulating the one or more syringes.
- 30 16. The system of claim 6 comprising a display for displaying image data of the injection area.
17. The system of claim 6 comprising an image analysis module for determining boundaries of objects within the injection area.

18. The system of claim 6 comprising an input device for receiving input from a user.
19. The system of claim 6 wherein the injection pipette orients the target blastocyst using a push-pull technique.
- 5 20. The system of claim 6 wherein the injection pipette orients the target blastocyst semi-automatically using a graphical user interface (GUI) and a user input device to vary the orientation of the target blastocyst.
21. The system of claim 6 wherein the injection pipette orients the target blastocyst automatically using image analysis.
- 10 22. The system of claim 6 wherein injection pipette injects one or more embryonic stem cells into the target blastocyst.
23. A system for facilitating automation of blastocyst microinjection, the system comprising:
 - 15 an integrated circuit having a plurality of channels forming a fluidic communication path for blastocysts and at least one blastocyst microinjection area in the fluidic communications path, the integrated circuit forming a suction hole in the blastocyst microinjection area for holding a target blastocyst in place;
 - 20 at least one electrode for electrorotating the target blastocyst so as to be suitable for injection;
 - a microelectromechanical (MEMS) needle for injecting one or more cells into the target blastocyst; and
 - a sensor for observing the position and orientation of the injection area.
- 25 24. The system of claim 23 wherein the sensor includes an optical sensor.
25. The system of claim 23 comprising a light source located opposite the injection area for illuminating the blastocyst.
26. The system of claim 23 comprising an input device for receiving input from a user.
- 30 27. The system of claim 23 wherein the at least one electrode orients the target blastocyst automatically using image analysis.

28. The system of claim 23 wherein injection pipette injects one or more embryonic stem cells into the target blastocyst.
29. A computer readable medium comprising computer executable instructions embodied in a tangible computer readable medium and
5 when executed by a processor of a computer performs steps comprising:
automatically feeding a target blastocyst from among a plurality of blastocysts into a blastocyst microinjection area;
automatically holding the target blastocyst in a fixed position
10 within the blastocyst microinjection area;
orienting the target blastocyst so as to be suitable for injection;
injecting one or more cells into the target blastocyst; and
automatically releasing and removing the target blastocyst from
the blastocyst microinjection area after injection of the one or more
15 cells.

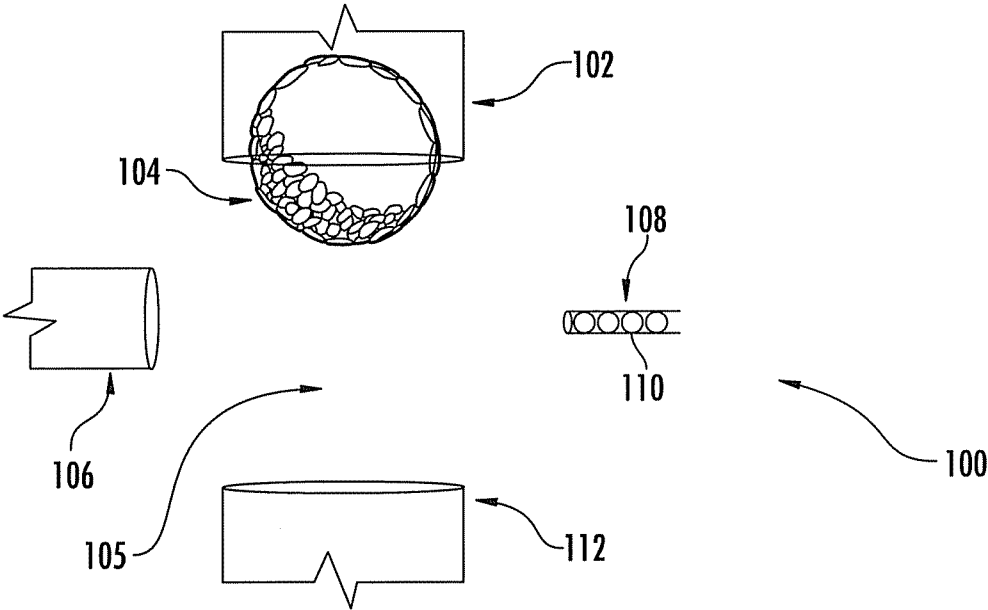


FIG. 1

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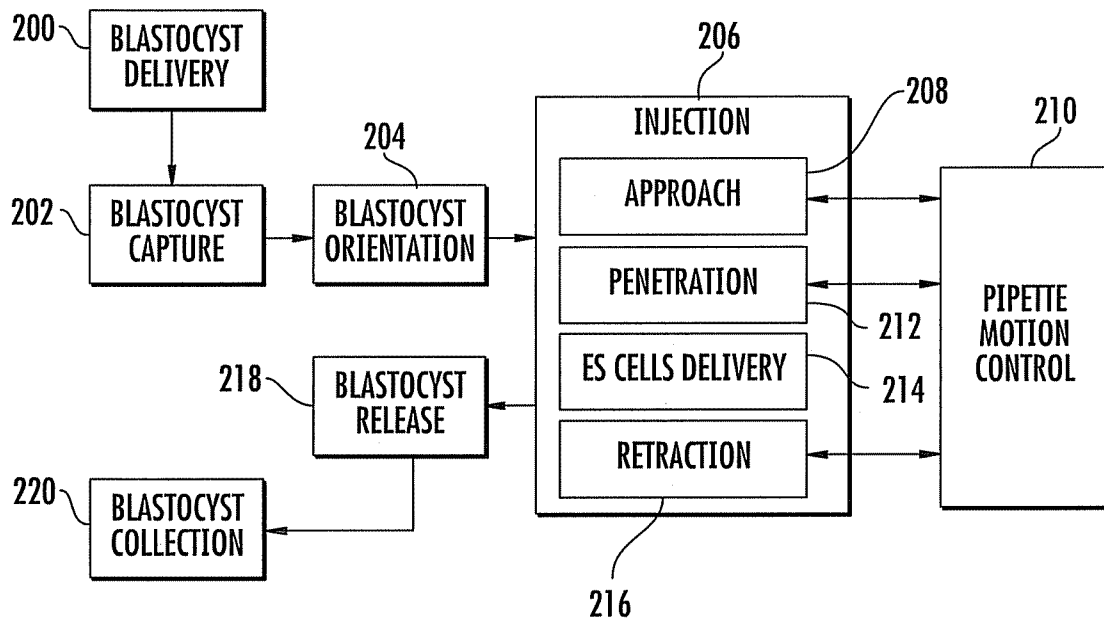


FIG. 2A

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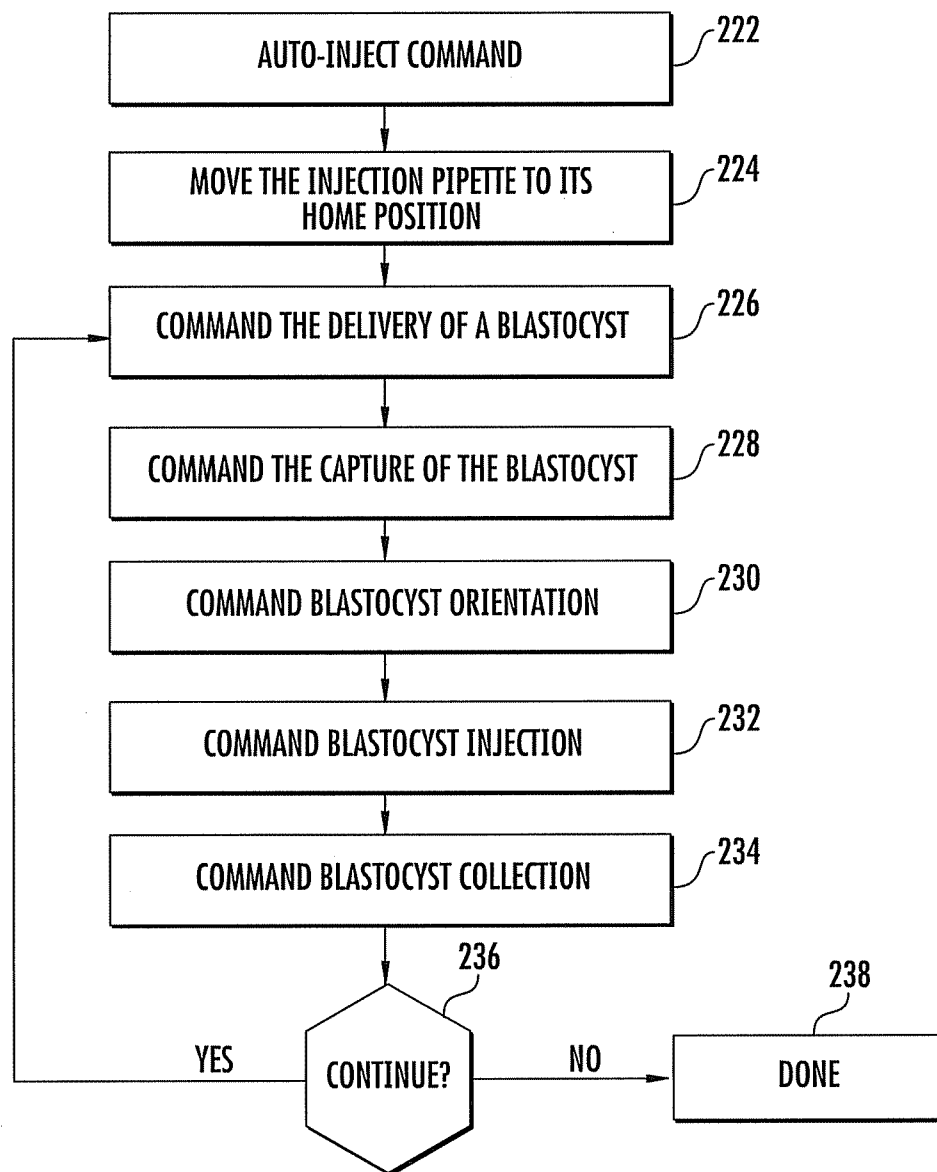


FIG. 2B

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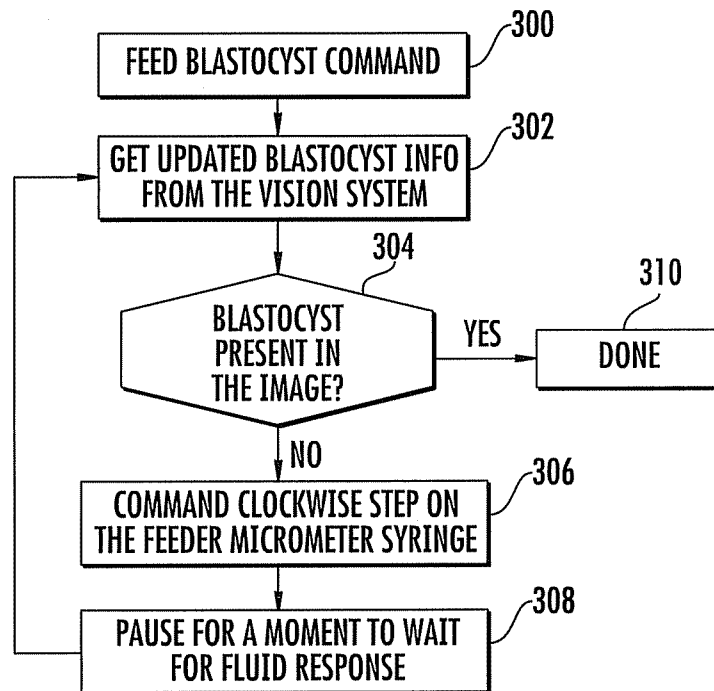


FIG. 3

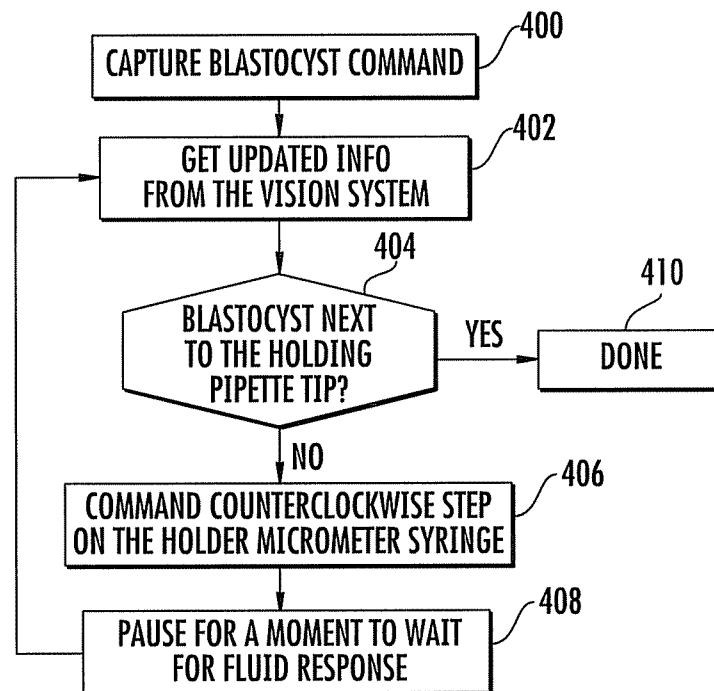


FIG. 4

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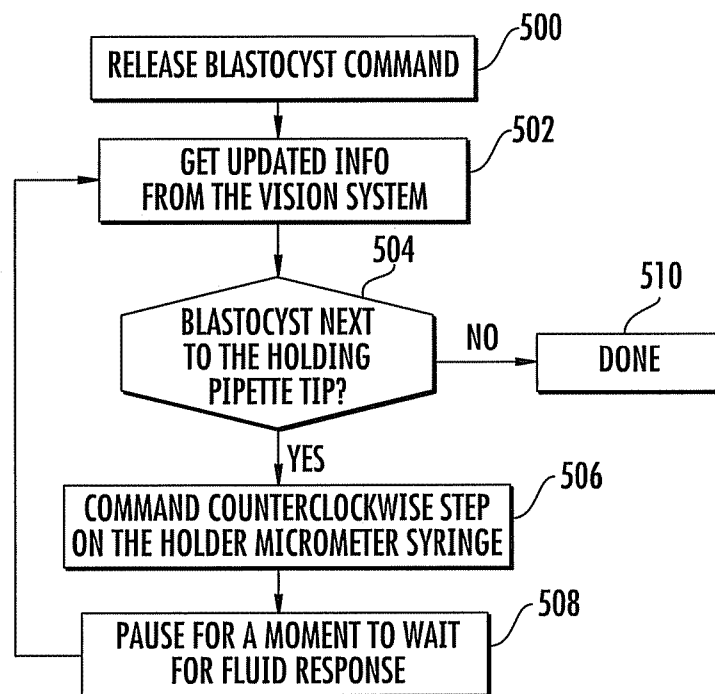


FIG. 5

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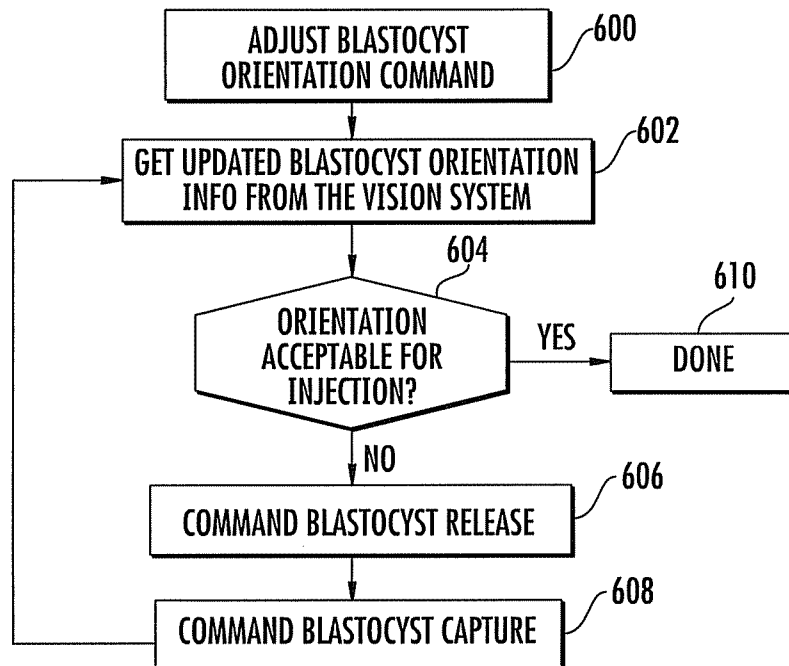


FIG. 6

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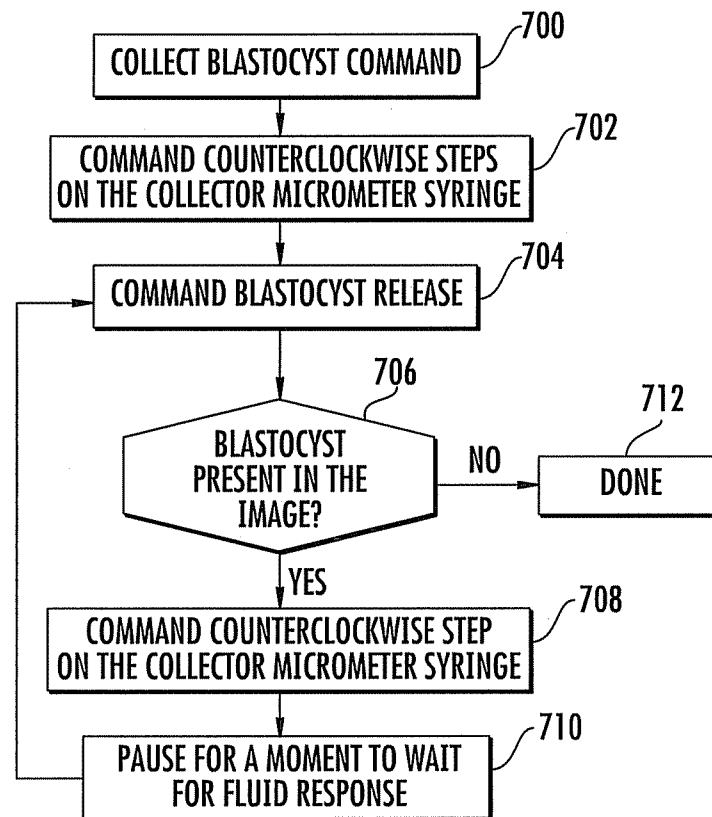


FIG. 7

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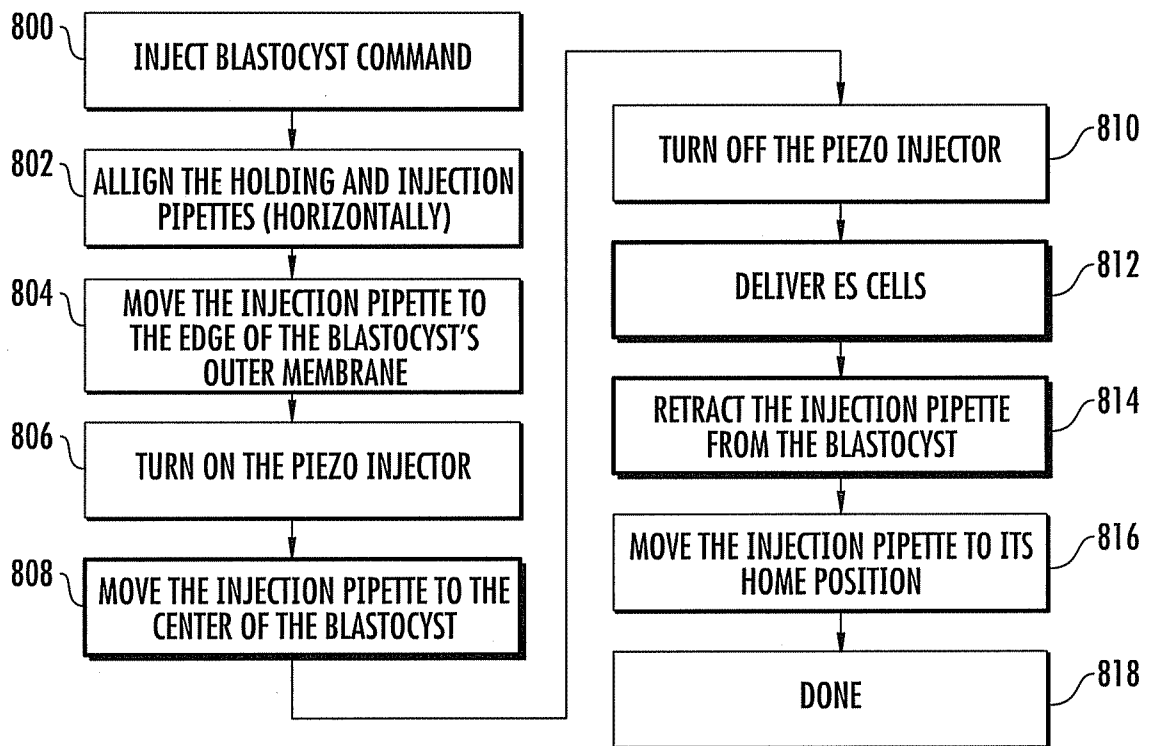


FIG. 8

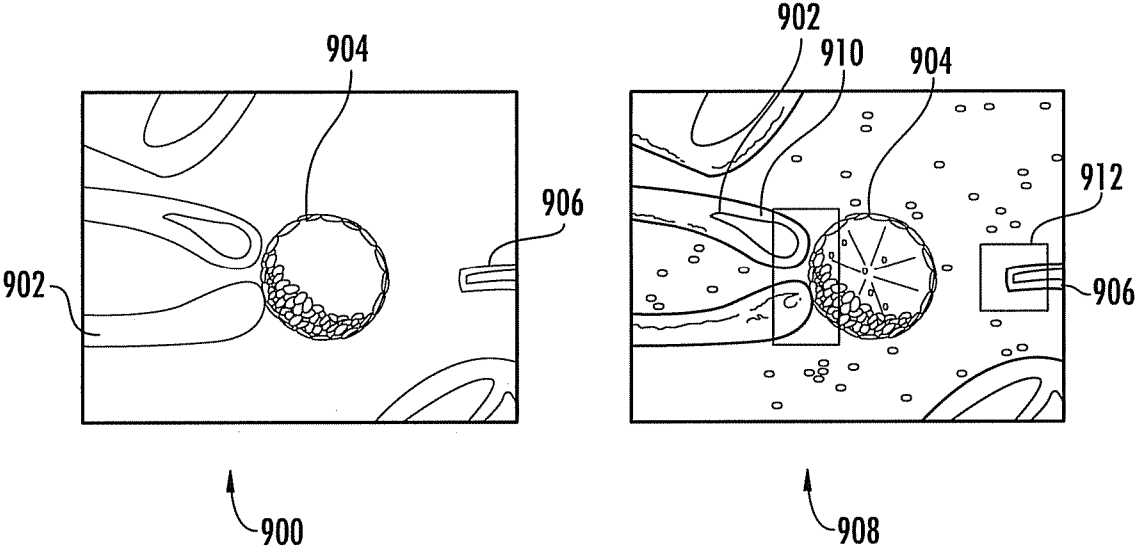
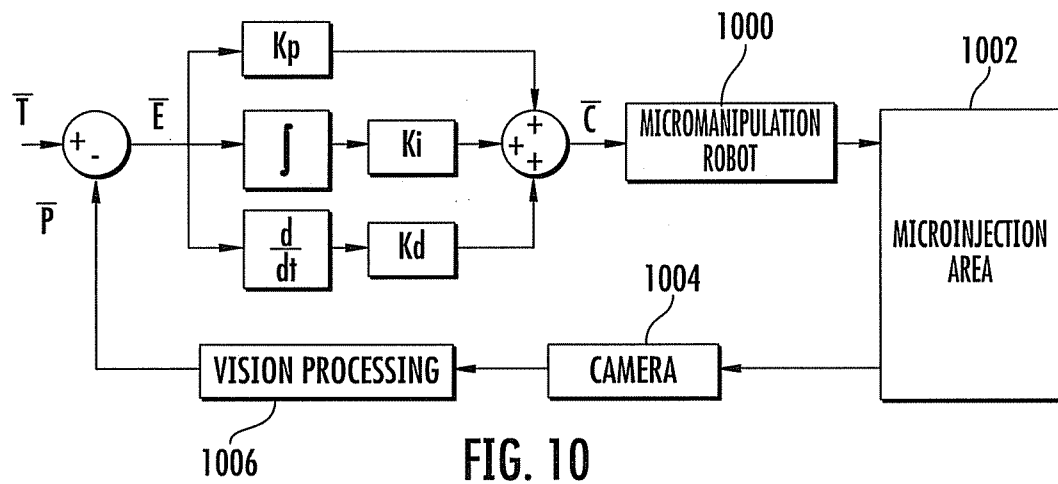


FIG. 9

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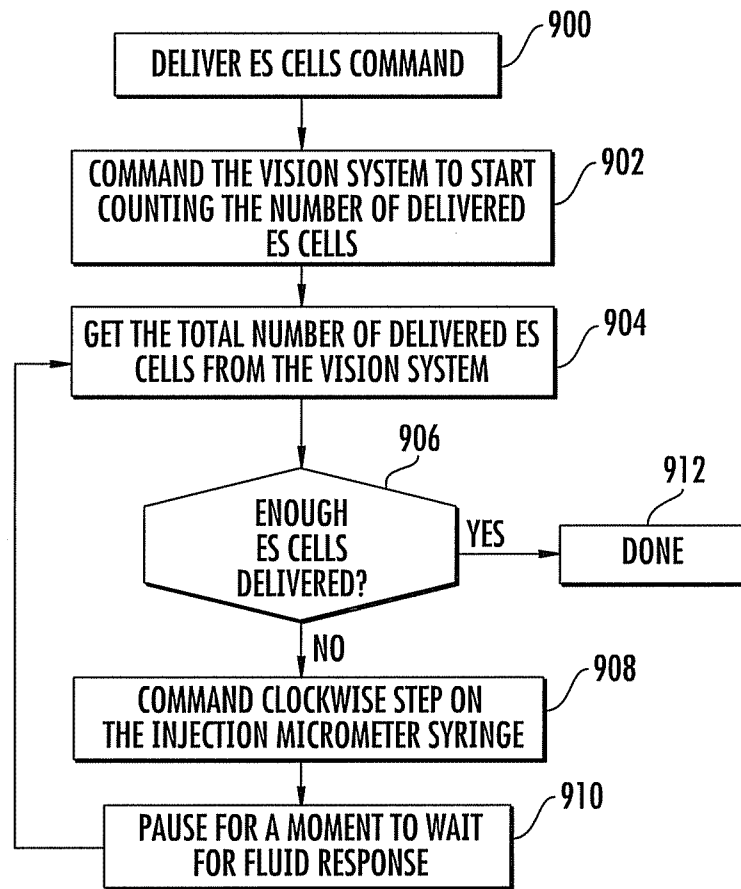


FIG. 11

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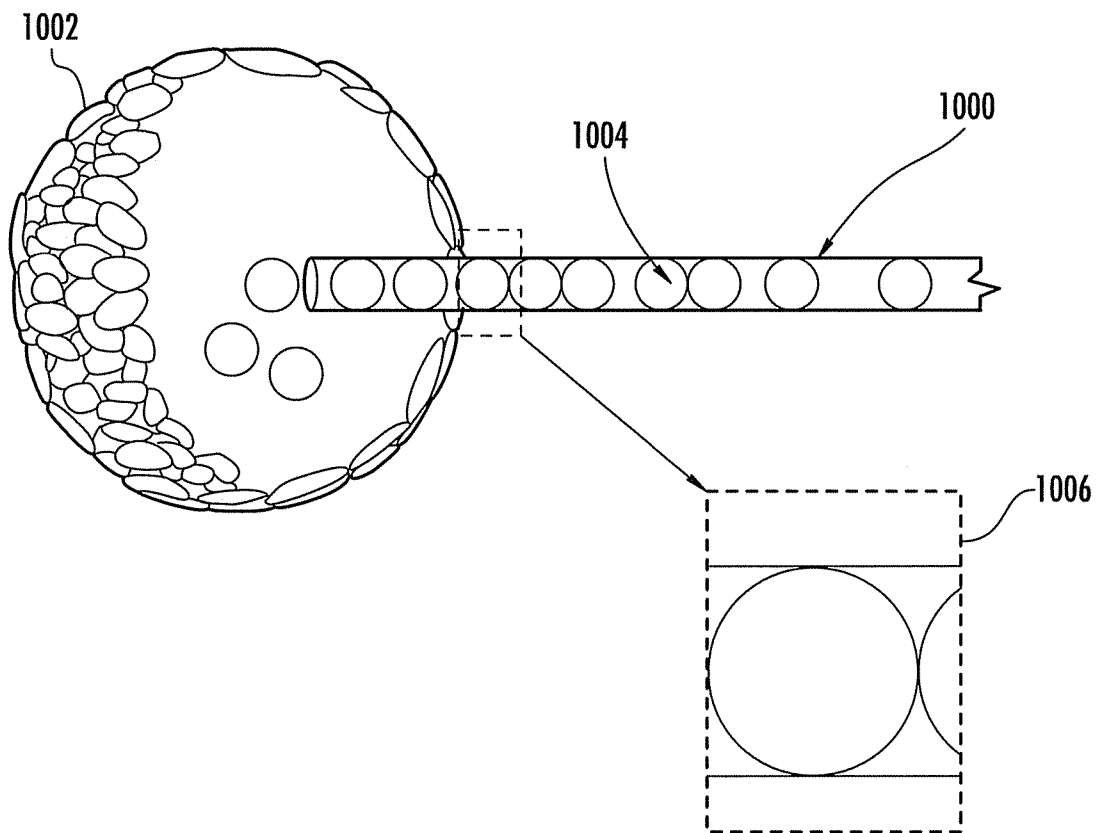


FIG. 12

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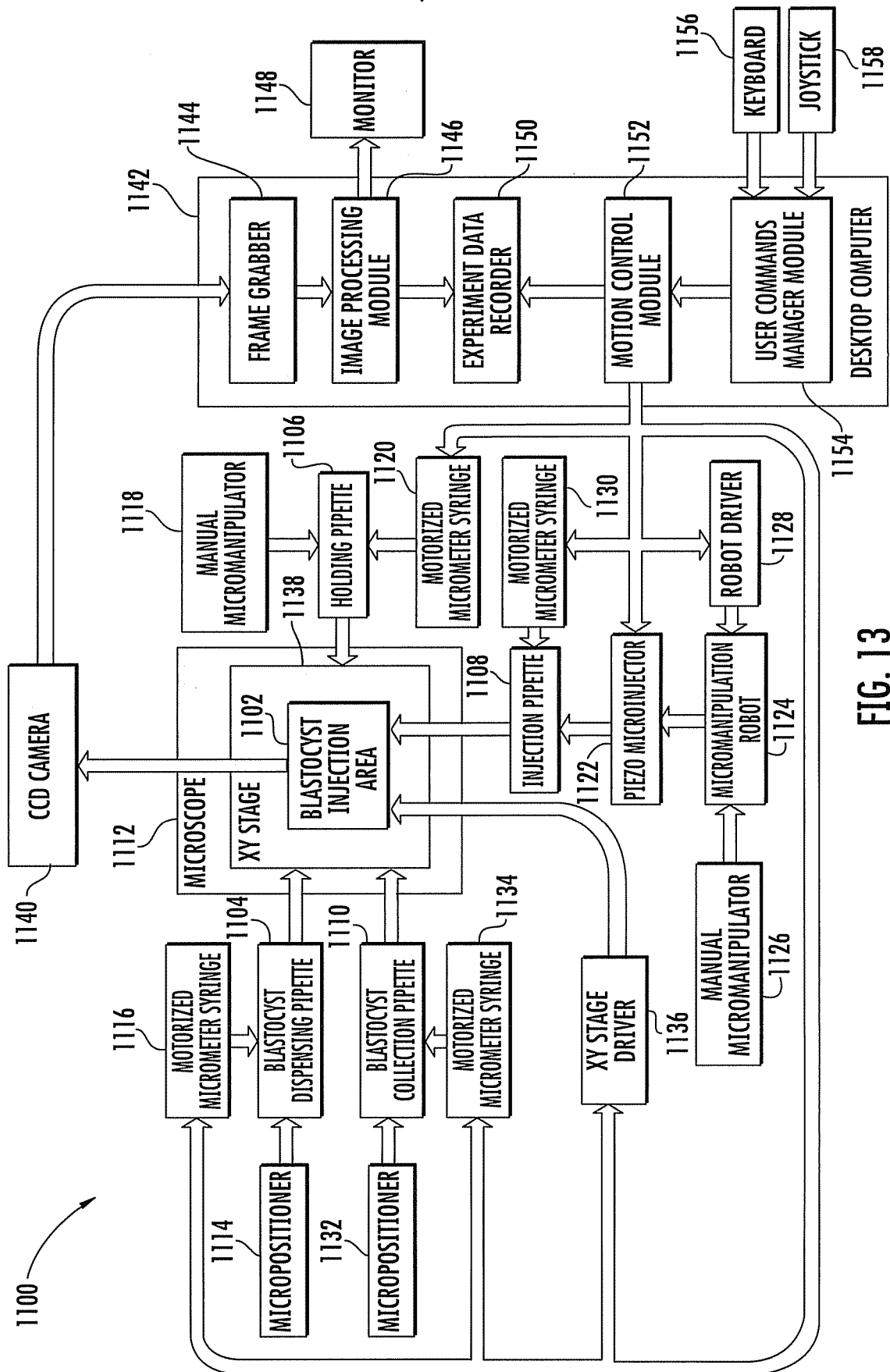


FIG. 13

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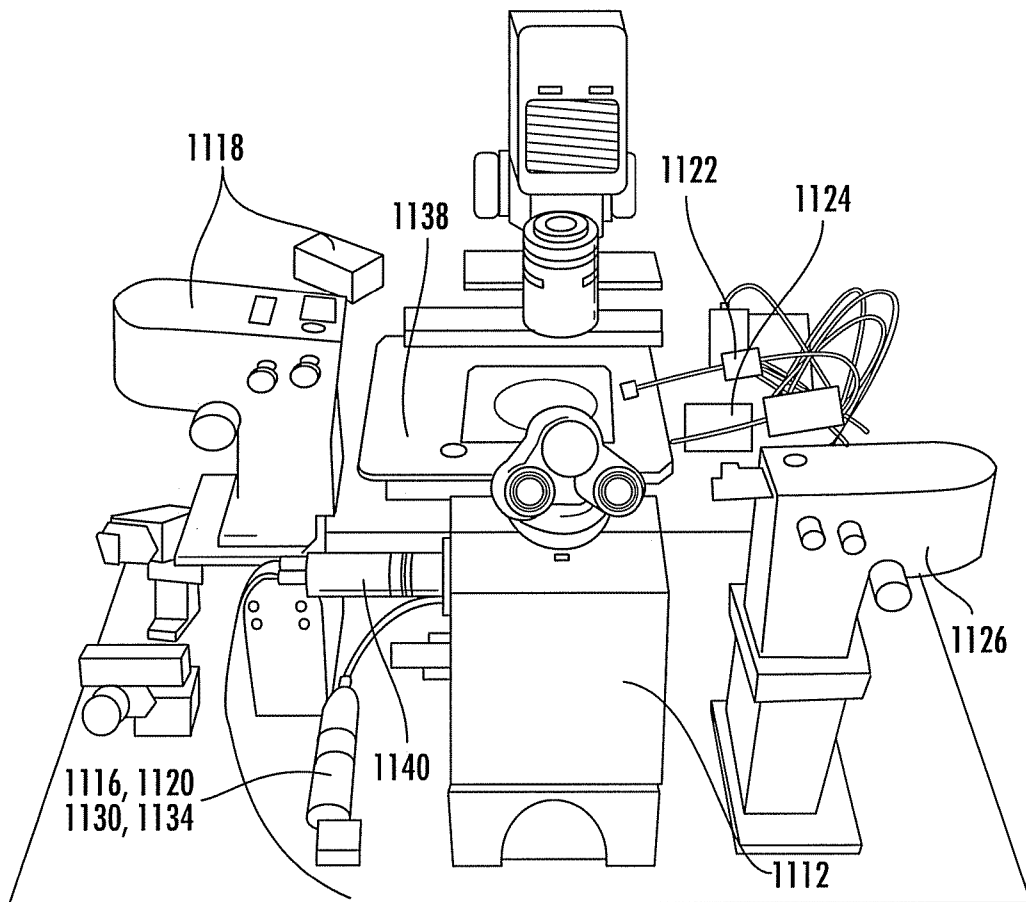


FIG. 14A

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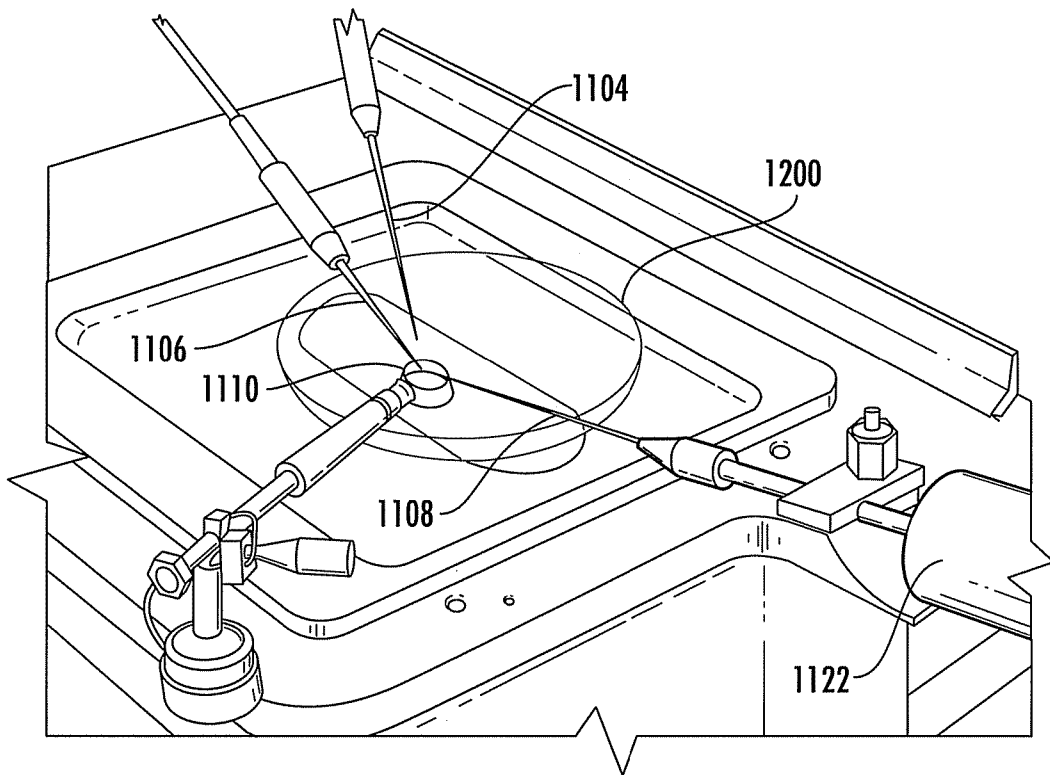


FIG. 14B

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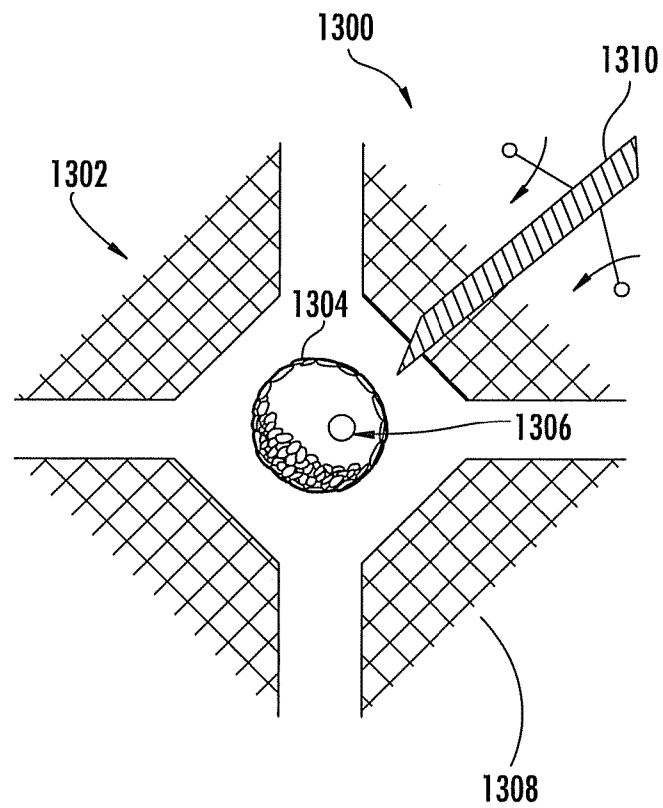


FIG. 15A

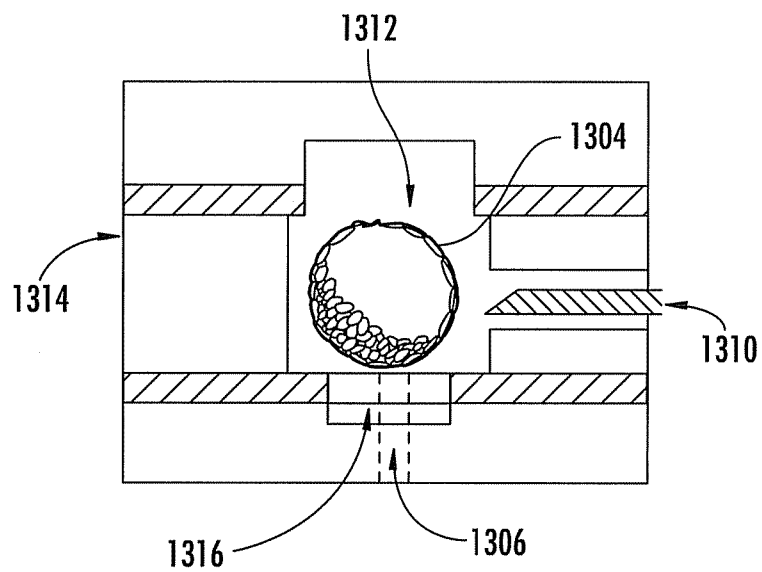


FIG. 15B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/086871**A. CLASSIFICATION OF SUBJECT MATTER***C12N 15/89(2006.01)i, C12M 1/26(2006.01)i*

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)

IPC: C12N 15/89, C12M 1/26

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

Korean Utility models and applications for Utility models since 1975
Japanese Utility models and applications for Utility models since 1975

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

eKIPASS(KIPO internal) & keywords: blastocyst, microinjection

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	KR 10-2005-0023483 A (SEOUL NATIONAL UNIVERSITY INDUSTRY FOUNDATION) 10 March 2005 See page 3, line 35 - page 12, line 7; and figures 1-10.	1 - 29
A	KR 10-2003-0009482 A (THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS) 29 January 2003 See page 4, line 12 - page 10, line 14; and figures 1-10.	1 - 29
A	JP 13-103867 A (NATL. FEDERATION OF AGRICULT. COOP. ASSOC. &, UNIV. OKAYAMA) 17 April 2001 See paragraph 4 - paragraph 30.	1 - 29
A	JP 17-529609 A (KOBENHAVNS AMTS SYGEHUS HERLEV) 06 October 2005 See the whole document.	1 - 29

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

12 MAY 2009 (12.05.2009)

Date of mailing of the international search report

12 MAY 2009 (12.05.2009)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, 139 Seonsa-ro, Seo-
gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

LEE, CHUNG HO

Telephone No. 82-42-481-8133



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2008/086871

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