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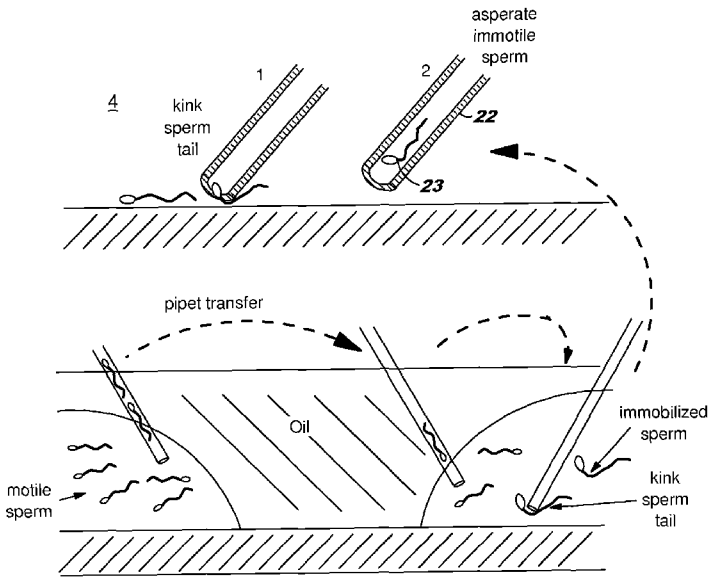
FIG. 3



(57) Abstract: A robotic microfluidic incubator system has a thin transparent sidewall and close proximity of the embryo/oocyte/cultured cells to the sidewall allow close approach of a side view microscope with adequate focal length for mid to high power. This arrangement permits microscopic examination of multiple culture wells when arranged in rows (linear or along the circumference of a carousel). Manual or automated side to side movement of the linear well row, or rotation of the carousel, allows rapid inspection of the contents each well. Automated systems with video capability also allow remote inspection of wells by video connection or Internet connection, and automated video systems can record oft-hours inspections or time lapse development in culture (i.e. embryo cell division progression, or axon growth in neuron cell cultures).

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AMENDED CLAIMS
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1. A micromanipulator system comprising:

a container;

said container including a stationary suction channel;

at least one actuator;

wherein various tools can be interchangeably connected to said actuator.

2. The micromanipulator system of claim 1 wherein the container and stationary suction channel are transparent.

3. The micromanipulator system of claim 1 or 2 wherein the container has multiple stationary suction channels in a group well.

4. The micromanipulator system of any of claims 1 to 3 having more than one actuator, wherein multiple tools can be used simultaneously.

5. A microfluidic chip system comprising:

at least one chip;

said chip having a receptacle for biological material;

said receptacle being transparent;

said receptacle including a well; and

said receptacle attachable to other receptacles.

6. The microfluidic chip system of claim 5 wherein more than one receptacle is permanently connected as a group.

7. The microfluidic chip system of claim 5 wherein more than one receptacle is removably connected as a group.

8. The microfluidic system of any of claims 5 to 7 wherein multiple groups of permanently attached receptacles are removably attached to one another.

9. The microfluidic system of any of claims 5 to 8 wherein the system is used to store biological material.

10. The microfluidic system of any of claims 5 to 9 wherein the system is used to culture biological material.

11. The microfluidic system of any of claims 5 to 10 wherein the system is used to manipulate biological material.

12. The microfluidic system of any of claims 5 to 11 wherein the system is used to observe biological material.

13. The microfluidic system of any of claims 5 to 12 wherein the system is used to freeze biological material.

14. The microfluidic system of any of claims 5 to 13 wherein the system is used to thaw biological material.

15. The microfluidic system of any of claims 5 to 14 wherein the receptacle contains more than one well.

16. The microfluidic system of any of claims 5 to 15 wherein the well has a holding vacuum channel.

17. The microfluidic system of any of claims 5 to 16 wherein the well has a side relief feature.

18. The microfluidic system of any of claims 5 to 17 wherein the chip has two or more microchannels leading to the biological material.

19. A culture media supply comprising:

a container;

a lid;

said lid having an aperture to receive at least one tube;

said lid having a gas exhaust port;

said lid having a second aperture to receive a gas concentration and temperature sensor;

a base; and

a feed port.

20. The culture media supply of claim 20 wherein the feed port is located on the base.

21. The culture media supply of claim 20 wherein the feed port is located on the lid.

22. The culture media supply of any of claims 20 to 21 wherein a filter is used to sterilize the media.

23. The culture media supply of any of claims 20 to 22 wherein a filter is used to remove bubbles from the media.

24. The culture media supply of any of claims 20 to 23 wherein sensors monitor and control gas concentration.

25. The culture media supply of any of claims 20 to 24 wherein sensors monitor and control gas concentration.

26. The culture media supply of any of claims 20 to 25 wherein media is transferred to the culture by gravity.

27. The culture media supply of any of claims 20 to 26 wherein media is transferred to the culture by capillary action.

28. The culture media supply of any of claims 20 to 27 wherein media is transferred to the culture by siphon.

29. The culture media supply of any of claims 20 to 28 wherein media is transferred to the culture by pump.

30. The culture media supply unit of any of claims 20 to 29 further comprising a mechanical fluid mixer inside the unit.

31. A method for supplying culture media comprising the steps of:

placing media in a container having a base, a lid containing at least one opening and at least one feed port;

altering the temperature of said media; and

inserting a first tube connected to a gas container in said aperture.

32. The method of claim 31 further comprising the steps of:

inserting a first end of a second tube into said feed port; and

attaching the second end of said second tube to a culture.

33. The method of claim 32 further comprising the step of sealing the media in the container.

34. The method of one of claims 32 to 33 wherein the temperature of the media is altered by electric element.

35. The method of one of claims 32 to 34 wherein the temperature of the media is altered by fluid jacket connection.

36. The method of one of claims 32 to 35 wherein the temperature of the media is altered by microwave.

37. The method of one of claims 32 to 36 wherein multiple units are used in parallel to provide customized gas and solute concentrations to a culture system.

38. The method of one of claims 32 to 37 wherein multiple units are used in series to provide customized gas and solute concentrations to a culture system.

39. A microfluidic chip incubation system comprising:

an incubation module;

the incubation module having at least one port and a fluid chamber;

a vaginal capsule;

a clip;

wherein said incubation module is placed inside said vaginal capsule and the clip is placed around the vaginal capsule.

40. The microfluidic chip incubation system of claim 39 further comprising a culture well.

41. The microfluidic chip incubation system of either of claims 39 or 40 further comprising a fluid trap.

42. A method for incubating embryos comprising:

inserting liquid culture media into a microfluidic chip;

inserting dissolved gas into the microfluidic chip;

placing at least one embryo into the microfluidic chip;

encasing the chip into a module;

sealing the module;

placing the module inside a patient;

removing the module at the end of an incubation period; and

removing the microfluidic chip from the module.

43. The method of claim 42 further comprising the step of placing the module in a vagina of the patient.

44. The method of either claim 42 or 43 further comprising the step of transferring an embryo to the patient's uterus.

45. The method of any of claims 42 to 44 further comprising the step of freezing an embryo for delayed uterine transfer.

46. A freezing stem comprising:

a microfluidic chip;

the chip having at least one port;

an extension from the chip;

the extension having a smaller width than the chip; and

at least one microchannel extending between the chip and the extension.

47. The freezing stem of claim 46 wherein the chip has two ports.

48. The freezing stem of either claim 46 or 47 wherein two microchannels extend between the chip and the extension.

49. The freezing stem of claim 48 wherein one microchannel is larger than the other.

50. The freezing stem of any of claims 46 to 49 wherein the chip is transparent.

51. The freezing stem of any of claims 46 to 49 wherein the chip is opaque.

52. The freezing stem of any of claims 46 to 51 wherein the extension is transparent.

53. The freezing stem of any of claims 46 to 51 wherein the extension is opaque.

54. The freezing stem of any of claims 46 to 53 further comprising a cap to cover the extension.

55. The freezing stem of any of claims 46 to 54 wherein the chip has multiple extensions.

56. The freezing stem of any of claims 46 to 55 wherein more than one chip is removably attached to a parallel media flow system.

57. The freezing stem of any of claims 46 to 56 wherein an extension is located on the base of the chip body.

58. The freezing stem of any of claims 46 to 57 wherein an extension is located on the side of the chip body.

59. The freezing stem of any of claims 46 to 58 wherein the port is sealed with a membrane penetrable by a needle.

60. The freezing stem of claim 59 where the membrane is resealable with adhesive.

61. A freezing system comprising:

a microfluidic chip;

the chip having at least one port;

the chip having at least one microchamber;

at least one microchannel extending between the port and the microchamber.

62. The freezing system of claim 61 wherein the chip has multiple ports.

63. The freezing system of either claim 61 or 62 wherein the chip has multiple microchambers.

64. The freezing system of any of claims 61 to 63 wherein the chip has multiple microchannels.

65. The freezing system of any of claims 61 to 64 wherein the chip is thinner at the microchamber.

66. The freezing system of any of claims 61 to 65 wherein microchambers are located in the approximate center of the chip.

67. The freezing system of any of claims 61 to 66 wherein microchambers are located at the edges of the chip.

68. The freezing system of any of claims 61 to 67 having ribs between microchambers.

69. A method for freezing a specimen comprising the steps of:

immersing the specimen in culture fluid or fluid droplet;

placing the specimen in a chip having a stem;

positioning the specimen at the tip of the stem;

rapidly plunging the chip into a freezing agent; and

storing the chip at a temperature within a few degrees of absolute zero.

70. The method of claims 69 wherein the freezing agent is cryogen.

71. The method of either of claims 69 or 70 wherein the chip is plunged into the freezing agent stem first.

72. The method of claim 69 further comprising the step of inserting an inert gas bubble in the culture fluid.

73. The method of either claim 69 or claim 72 further comprising the step of adding cryoprotective solution.

74. The method of any of claims 69 to 73 further comprising the step of performing a cell culture on the specimen before freezing.

75. The method of any of claims 69 to 74 further comprising the step of thawing the specimen.

76. The method of claim 75 wherein the specimen is thawed by rapidly plunging the chip into warm water.

77. The method of either claim 75 or 76 wherein the specimen is thawed by exposure to radiant heat.

78. The method of any of claims 75 to 77 wherein the specimen is thawed by exposure to microwave.

79. The method of any of claims 73 to 78 further comprising the step of diluting the cryoprotective solution.

80. The method of any of claims 74 to 79 further comprising the step of diluting the cell culture of the specimen.

81. The method of any of claims 75 to 80 further comprising the step of retrieving the specimen from the stem.

82. A microfluidic sperm separation network comprising:

a sperm solution entry port;

a sperm solution exit port;

a media entry port;

at least one network feed channel;

a series of connected microchannels; and

multiple product exit ports.

83. The sperm separation of claim 82 further comprising at least one gradient solution entry port.

84. The sperm separation network of claim 83 wherein a single gradient entry port and single media entry port feed into a large chamber which terminates in parallel microchannels.

85. The sperm separation network of claim 82 having multiple gradient solution entry ports.

86. The sperm separation network of either of any of claims 82 to 85 having automated mixers in the microchannels.

87. The sperm separation network of any of claims 82 to 86 wherein the gradient solution comprises an albumin solution.

88. The sperm separation network of any of claims 82 to 86 wherein the gradient solution comprises chemotactic agents.

89. The sperm separation network of any of claims 82 to 86 wherein the gradient solution comprises pH gradients.

90. The sperm separation network of any of claims 82 to 86 wherein the gradient solution comprises a sugar gradient.

91. The sperm separation network of any of claims 82 to 86 wherein the gradient solution comprises a carbohydrate gradient.

92. The sperm separation network of any of claims 82 to 86 wherein the gradient solution comprises a Percoll density gradient.

93. The sperm separation network of any of claims 82 to 92 wherein any of the microchannels has a side channel.

94. The sperm separation network of any of claims 82 to 93 wherein the network is incorporated onto a single microfluidic chip.

95. The sperm separation network of any of claims 82 to 93 wherein two or more plates are fused together with active channels engraved in each.

96. The sperm separation network of claim 95 wherein the entry ports are located on one plate and the exit ports are located on a separate plate.

97. The sperm separation network of any of claims 82 to 96 wherein the sperm separation network is looped and continuously flowing.

98. A method for separating sperm comprising the steps of:

creating a laminar flow system comprised of a sperm solution entry port, a sperm solution exit port, a media entry port, at least one network feed channel; a series of connected microchannels, and multiple product exit ports;

inserting media into said laminar flow system;

placing sperm solution in said laminar flow system; and

applying a gradient to the laminar flow system.

99. The method of claim 98 wherein a force gradient is used.

100. The method of claim 98 or 99 wherein the gradient is created using thermal force.

101. The method of claim 98 wherein the gradient is created using an electric field.

102. The method of claim 98 wherein the gradient is created using a magnetic field.

103. The method of claim 98 wherein the gradient is created using centripetal force.

104. The method of claim 98 further comprising the step of adding a gradient solution entry port.

105. The method of claim 98 further comprising the step of adding multiple gradient solution entry ports.

106. The method of claim 104 further comprising the step of adding gradient solution and media solution, wherein the gradient solution and media solution feed into a large chamber which terminates in parallel microchannels.

107. The method of claim 105 further comprising the step of adding gradient solution having at least two different concentrations.

108. The method of either of claims 106 or 107 wherein the gradient solution comprises an albumin solution.

109. The method of either of claims 106 or 107 wherein the gradient solution comprises chemotactic agents.

110. The method of either of claims 106 or 107 wherein the gradient solution comprises pH gradients.

111. The method of either of claims 106 or 107 wherein the gradient solution comprises a sugar gradient.

112. The method of either of claims 106 or 107 wherein the gradient solution comprises a carbohydrate gradient.

113. The method of either of claims 106 or 107 wherein the gradient solution comprises a Percoll density gradient.

114. The method of any of claims 98 to 113 wherein the laminar flow system is looped and continuously flowing.

115. A stripping method for use with an oocyte having a cumulus mass, and for use with a specimen microchannel, the microchannel having a stripping channel communicating therewith and transverse thereto, the stripping channel being too narrow to permit passage of the oocyte therethrough, the stripping channel defining first and second positions within the microchannel on first and second sides of the stripping channel and adjacent thereto, the method comprising the steps of:

inserting the oocyte with the cumulus mass into a chip well;

drawing the oocyte with the cumulus mass through a funnel into a specimen microchannel to the first position;

pumping a cumulus digestive enzyme rapidly and alternately back and forth along the stripping channel and removing some of the cumulus mass away from the oocyte;

disposing of some fragments of the removed cumulus mass through the stripping channel;

stopping the pumping of the cumulus digestive enzyme;

pumping fluid to or from the specimen channel so as to move the oocyte to the second position;

pumping additional cumulus digestive enzyme rapidly and alternately back and forth along the stripping channel and removing some more of the cumulus mass away from the oocyte;

disposing of some fragments of the removed cumulus mass through the stripping channel; and

stopping the pumping of the cumulus digestive enzyme.

116. The method of claim 115 further comprising the steps of:

pumping fluid to or from the specimen channel so as to move the oocyte back to the first position;

pumping additional cumulus digestive enzyme rapidly and alternately back and forth along the stripping channel and removing some more of the cumulus mass away from the oocyte;

disposing of some fragments of the removed cumulus mass through the stripping channel; and

stopping the pumping of the cumulus digestive enzyme.

117. The method of claim 115 further comprising the steps of:

pumping fluid to or from the specimen channel so as to move the oocyte back to at least the funnel;

rotating the oocyte by means of additional fluid flow;

pumping fluid to or from the specimen channel so as to move the oocyte back to the first or second position;

pumping additional cumulus digestive enzyme rapidly and alternately back and forth along the stripping channel and removing some more of the cumulus mass away from the oocyte;

disposing of some fragments of the removed cumulus mass through the stripping channel; and

stopping the pumping of the cumulus digestive enzyme.

118. The method of claim 115 wherein the cumulus digestive enzyme is hyanourandase.

119. The method of claim 115 further characterized in that the specimen channel is curved, whereby physical bending stress is applied to the cumulus mass when the cumulus mass passes through the curve.

120. A stripping method for use with an oocyte having a cumulus mass, and for use with a specimen microchannel, the microchannel having first and second stripping channels each communicating therewith and each transverse thereto, each stripping channel being too narrow to permit passage of the oocyte therethrough, the first and second stripping channels spaced apart sufficiently to permit an oocyte to be positioned therebetween; the method comprising the steps of:

inserting the oocyte with the cumulus mass into a chip well;

drawing the oocyte with the cumulus mass through a funnel into a specimen microchannel to a position between the first and second stripping channels;

pumping a cumulus digestive enzyme rapidly and alternately back and forth along each of the stripping channels and removing some of the cumulus mass away from the oocyte;

disposing of some fragments of the removed cumulus mass through the stripping channels;

stopping the pumping of the cumulus digestive enzyme.

121. The method of claim 120 further comprising the steps of:

pumping fluid to or from the specimen channel so as to move the oocyte back to at least the funnel;

rotating the oocyte by means of additional fluid flow;

pumping fluid to or from the specimen channel so as to move the oocyte back to the position between the first and second stripping channels;

pumping additional cumulus digestive enzyme rapidly and alternately back and forth along each of the stripping channels and removing some more of the cumulus mass away from the oocyte;

disposing of some fragments of the removed cumulus mass through the stripping channels; and

stopping the pumping of the cumulus digestive enzyme.

122. The method of claim 120 wherein the cumulus digestive enzyme is hyanourandase.

123. The method of claim 120 wherein the first and second stripping channels are parallel in the regions nearby to the specimen microchannel.

124. The method of claim 120 wherein the pumping of the cumulus digestive enzyme rapidly and alternately back and forth along each of the stripping channels is carried out simultaneously in the two stripping channels.

125. The method of claim 120 wherein the pumping of the cumulus digestive enzyme rapidly and alternately back and forth along each of the stripping channels is carried out simultaneously in the two stripping channels.

126. The method of claim 120 wherein the pumping of the cumulus digestive enzyme rapidly and alternately back and forth along each of the stripping channels is carried out in the same direction simultaneously in the two stripping channels.

127. The method of claim 120 wherein the pumping of the cumulus digestive enzyme rapidly and alternately back and forth along each of the stripping channels is carried out in the opposite direction simultaneously in the two stripping channels.

128. The method of either of claims 115 or 120 further comprising the step of fertilizing the oocyte.

129. The method of either of claims 115 or 120 wherein the oocyte is a human oocyte.

130. Apparatus for use in stripping an oocyte comprising:

a transparent solid cell,

the cell defining a specimen microchannel, the microchannel sized to permit passage of an oocyte with a cumulus mass;

the cell further defining a stripping channel communicating with the microchannel and transverse thereto, the stripping channel being too narrow to permit passage of the oocyte therethrough;

the cell further defining a funnel at one end of the microchannel;

the apparatus further comprising means for pumping a cumulus digestive enzyme rapidly and alternately back and forth along the stripping channel;

the apparatus further comprising means for pumping fluid to and from the specimen channel, whereby the oocyte with the cumulus mass may move therealong.

131. Apparatus for use in stripping an oocyte comprising:

a transparent solid cell,

the cell defining a specimen microchannel, the microchannel sized to permit passage of an oocyte with a cumulus mass;

the cell further defining first and second stripping channels communicating with the microchannel and transverse thereto, each stripping channel being too narrow to permit passage of the oocyte therethrough;

the first and second stripping channels spaced apart sufficiently to permit an oocyte to be positioned therebetween;

the cell further defining a funnel at one end of the microchannel;

the apparatus further comprising means for pumping a cumulus digestive enzyme rapidly and alternately back and forth along each stripping channel;

the apparatus further comprising means for pumping fluid to and from the specimen channel, whereby the oocyte with the cumulus mass may move therealong.

132. The apparatus of claim 130 or 131 wherein the oocyte is a human oocyte.

133. The apparatus of claim 131 wherein the first and second stripping channels are parallel in the regions nearby to the specimen microchannel.

134. A method for use with a specimen, and for use in an environment having gravity defining upward and downward directions, and for use relative to a horizontal surface having a suction port microchannel located below the horizontal surface, the suction port microchannel being too narrow to permit passage of the specimen therethrough; the method comprising the steps of:

providing a liquid medium above the horizontal surface;

providing a specimen within the liquid medium;

holding the specimen on the horizontal surface by means of suction at the suction port microchannel;

providing a micromanipulation tool manipulated by a microactuator, the microactuator in air and not within the liquid medium;

moving the micromanipulation tool downwards through the air and through the surface of the liquid medium to approach and contact the top of the specimen.

135. The method of claim 134 wherein the specimen is an oocyte.

136. The method of claim 134 wherein the specimen is an embryo.

137. The method of claim 135 or 136 wherein the specimen is from a human organ.

138. Apparatus for use with a specimen, the apparatus for use in an environment having gravity defining upward and downward directions, the apparatus comprising:

a horizontal surface;

above the horizontal surface, means for holding a liquid medium;

the apparatus defining a suction port microchannel located below the horizontal surface, the suction port microchannel being too narrow to permit passage of the specimen therethrough;

suction means coupled with the suction port microchannel;

a microactuator in air and not within the liquid medium;

a micromanipulation tool manipulated by the microactuator and disposed to be moved downward through the air toward the suction port microchannel.

139. The apparatus of claim 138 further comprising a microscope having an observation path from a side thereof.

140. A method for use with a specimen, and for use in an environment having gravity defining upward and downward directions, and for use relative to a horizontal surface having first and second suction port microchannels located below the horizontal surface, each

suction port microchannel being too narrow to permit passage of the specimen therethrough; the method comprising the steps of:

providing a liquid medium above the horizontal surface;

providing a specimen within the liquid medium;

holding the specimen on the horizontal surface by means of suction at the first suction port microchannel;

providing a micromanipulation tool manipulated by a microactuator, the microactuator in air and not within the liquid medium;

moving the micromanipulation tool downwards through the air and through the surface of the liquid medium to approach and contact the top of the specimen;

withdrawing the micromanipulation tool;

releasing the specimen by releasing the suction at the first suction port microchannel;

drawing the specimen to the second port microchannel by means of suction at the second suction port microchannel; and

releasing the specimen by releasing the suction at the second suction port microchannel.

141. The method of claim 140 wherein the specimen is an oocyte.

142. The method of claim 140 wherein the specimen is an embryo.

143. The method of claim 141 or 142 wherein the specimen is from a human organ.

144. Apparatus for use with a specimen, the apparatus for use in an environment having gravity defining upward and downward directions, the apparatus comprising:

a horizontal surface;

above the horizontal surface, means for holding a liquid medium;

the apparatus defining first and second suction port microchannels located below the horizontal surface, each suction port microchannel being too narrow to permit passage of the specimen therethrough;

respective suction means coupled with each of the suction port microchannels;

a microactuator in air and not within the liquid medium;

a micromanipulation tool manipulated by the microactuator and disposed to be moved downward through the air toward the suction port microchannels.

145. The apparatus of claim 144 further comprising a microscope having an observation path from a side thereof.

146. A sperm separation system comprising:

first, second, and third channels extended along a first direction;

the first and second channels passing adjacent to each other in a first shared region;

the second and third channels passing adjacent to each other in a second shared region;

the dimensions of the channels and shared regions such that fluid flow therewithin has a low Reynolds number and has laminar flow;

gradient means disposed relative to the first, second, and third channels, the gradient means selectively urging sperm from the first channel to the second channel and from the second channel to the third channel.

147. A sperm separation system comprising:

first, second, and third channels extended along a first direction;

the first and second channels passing adjacent to each other in a plurality of first shared regions;

the second and third channels passing adjacent to each other in a plurality of second shared regions;

the first shared regions alternating along the second channel with the second shared regions;

the dimensions of the channels and shared regions such that fluid flow therewithin has a low Reynolds number and has laminar flow; and

gradient means disposed relative to the first, second, and third channels, the gradient means selectively urging sperm from the first channel to the second channel and from the second channel to the third channel.

148. The system of claim 147 wherein flow along the first channel in the first direction recirculates through the first channel;

wherein flow along the second channel in the first direction recirculates through the second channel; and

wherein flow along the third channel in the first direction recirculates through the third channel.

149. The system of claims 146 or 147 wherein the gradient means is selected from the set consisting of albumin concentration, chemotactic agents, pH gradient, sugar gradient, carbohydrate gradient, Percoll density gradient, thermal gradient, electric-field gradient, magnetic gradient, and centrifugal force gradient.

150. A sperm separation method for use with first, second, and third channels extended along a first direction; the first and second channels passing adjacent to each other in a plurality of first shared regions; the second and third channels passing adjacent to each other in a plurality of second shared regions; the first shared regions alternating along the second channel with the second shared regions; the dimensions of the channels and shared regions such that fluid flow therewithin has a low Reynolds number and has laminar flow; the method comprising the steps of:

passing sperm in a liquid medium through the first, second, and third channels in the first direction;

applying a gradient relative to the first, second, and third channels, the gradient means selectively urging sperm from the first channel to the second channel and from the second channel to the third channel.

151. The method of claim 150 wherein flow along the first channel in the first direction recirculates through the first channel;

wherein flow along the second channel in the first direction recirculates through the second channel; and

wherein flow along the third channel in the first direction recirculates through the third channel.

152. The method of claim 150 wherein the applied gradient is selected from the set consisting of albumin concentration, chemotactic agents, pH gradient, sugar gradient, carbohydrate gradient, Percoll density gradient, thermal gradient, electric-field gradient, magnetic gradient, and centrifugal force gradient.