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(54) **HYBRID PRINTING PLATFORM FOR 3D BIOPRINTING OF LIVE ORGANS**

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B33Y 70/00	(2006.01)
A61F 2/02	(2006.01)

(52) **U.S. Cl.**

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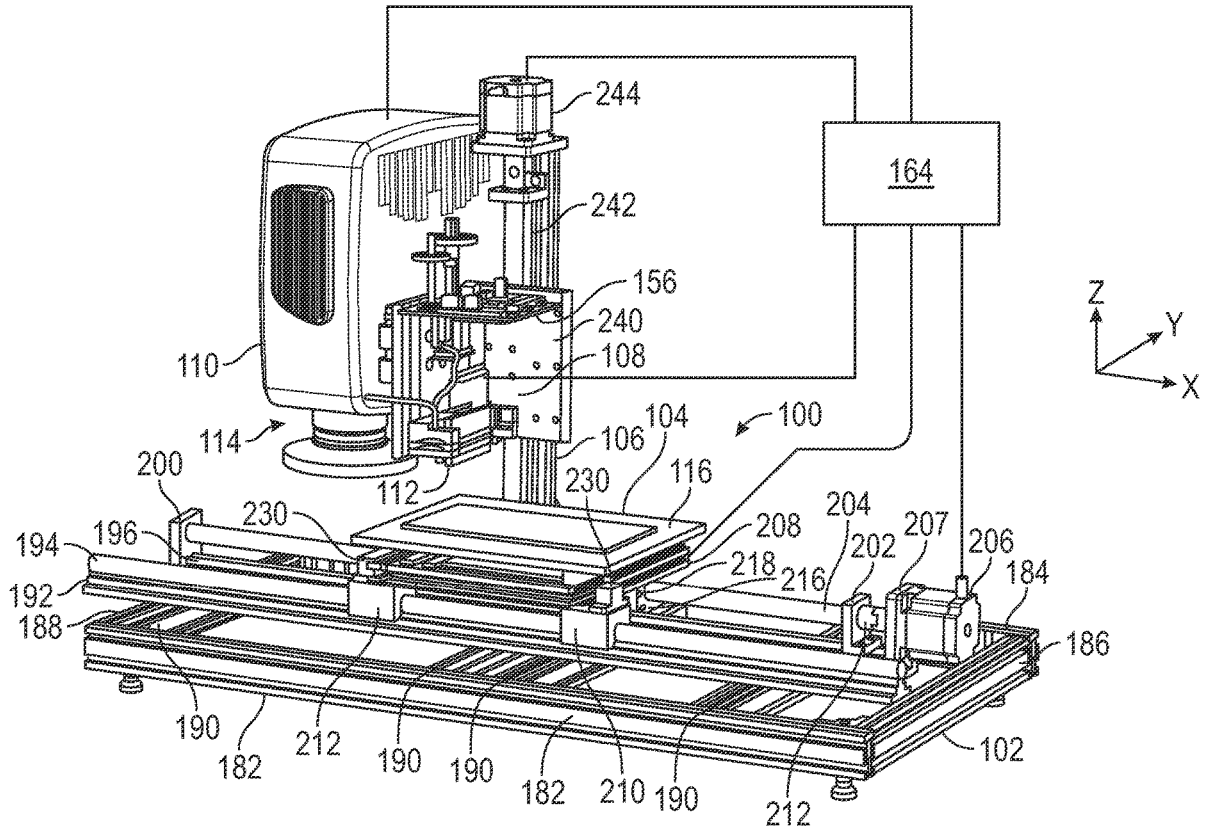
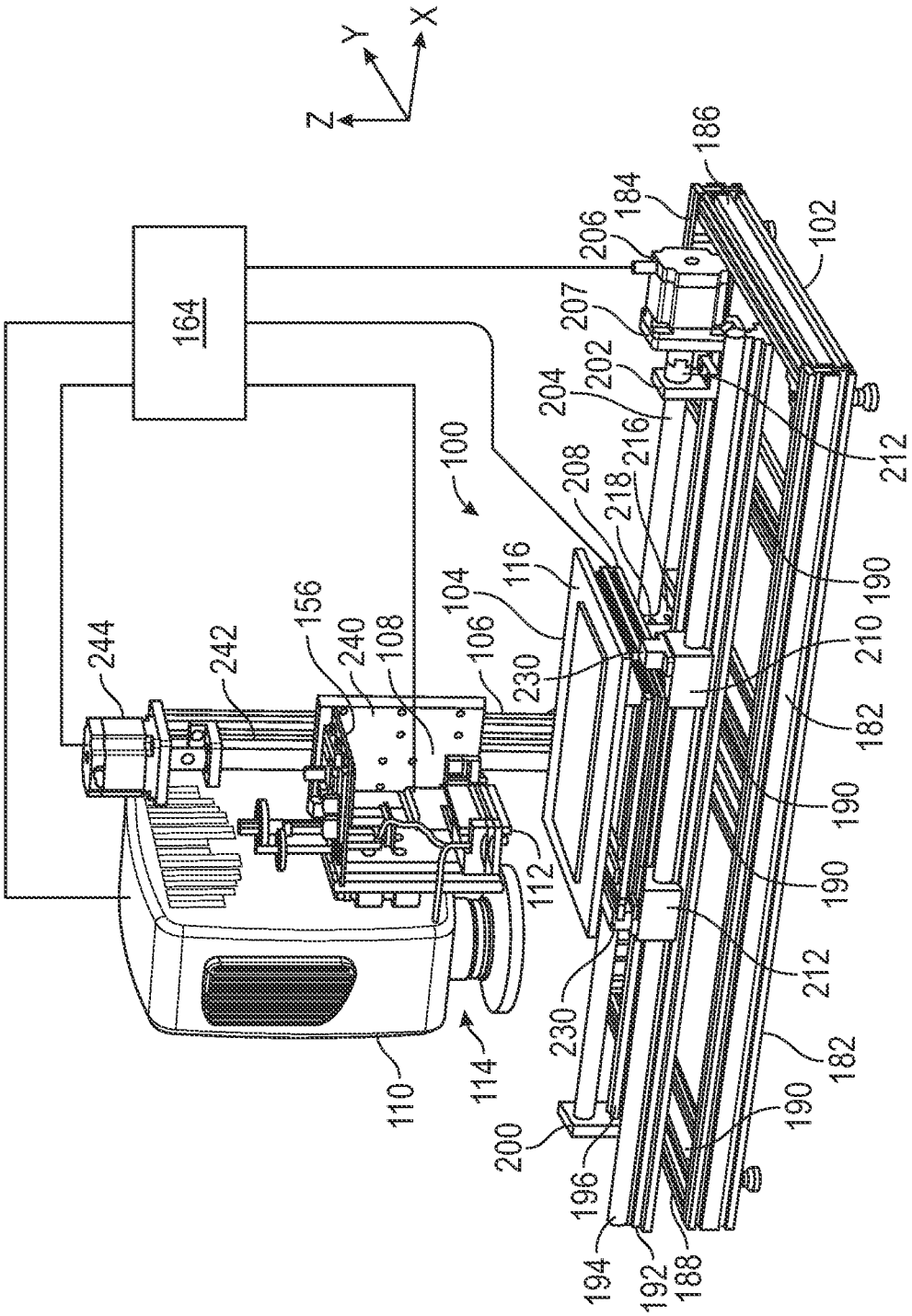
(57) **ABSTRACT**

A method of forming a three dimensional object includes dispensing droplets of an electromagnetic energy curable liquid onto a surface to form a plurality of layers of the three dimensional object in liquid form, wherein each droplet forms a layer of liquid on the surface which is larger than a minimum feature size of a structure to be formed by curing the curable liquid, and directing electromagnetic energy capable of curing the liquid and having a beam width intersecting the layer of liquid which is at least as small as the smallest feature of a structure to be formed in the curable liquid.




Z
Y
X
164
100
102
104
106
108
110
112
114
116
156
182
184
186
188
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192
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196
200
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207
208
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216
218
230
240
242
244

FIG. 1

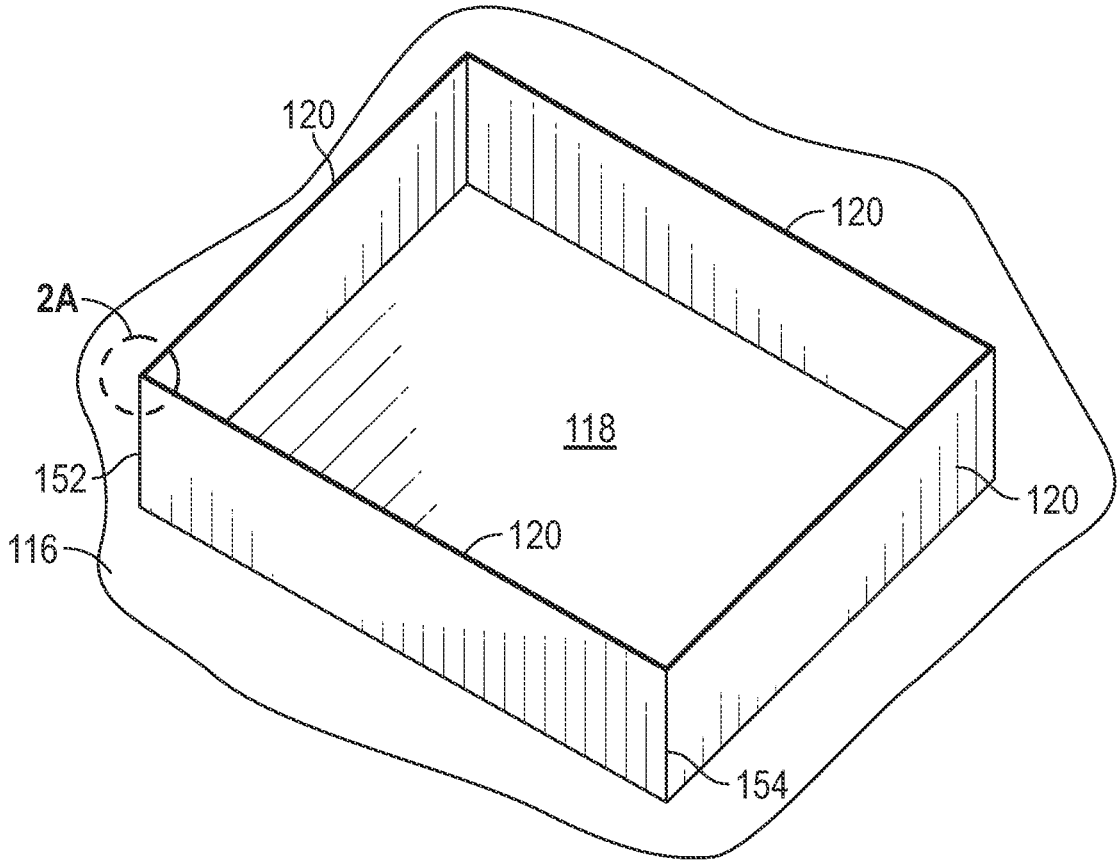

FIG. 2

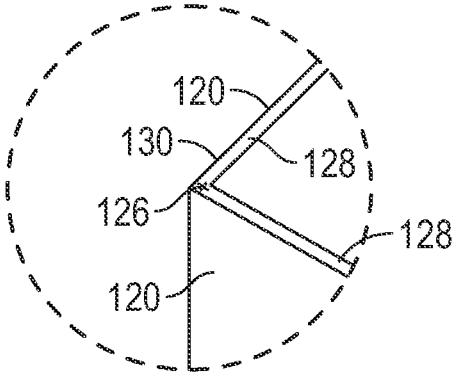

FIG. 2A

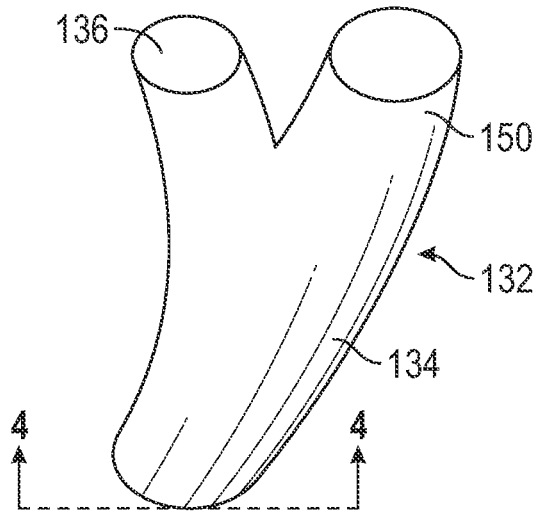

FIG. 3

FIG. 4

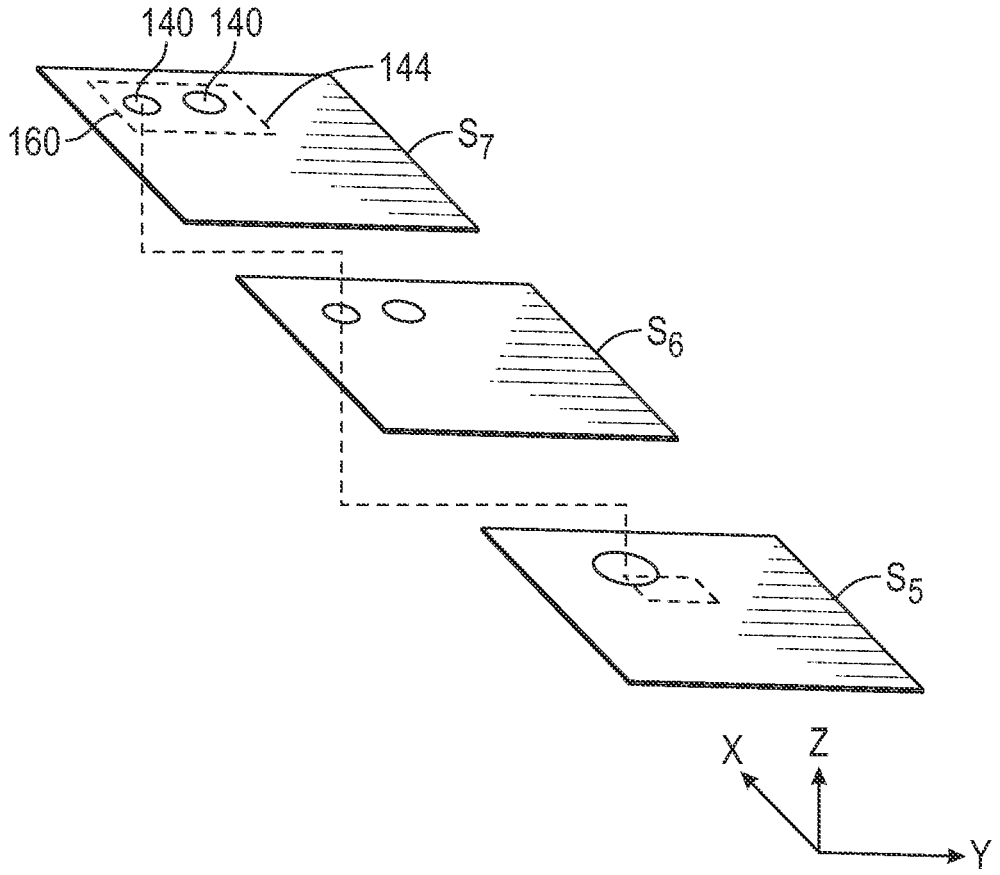

FIG. 5

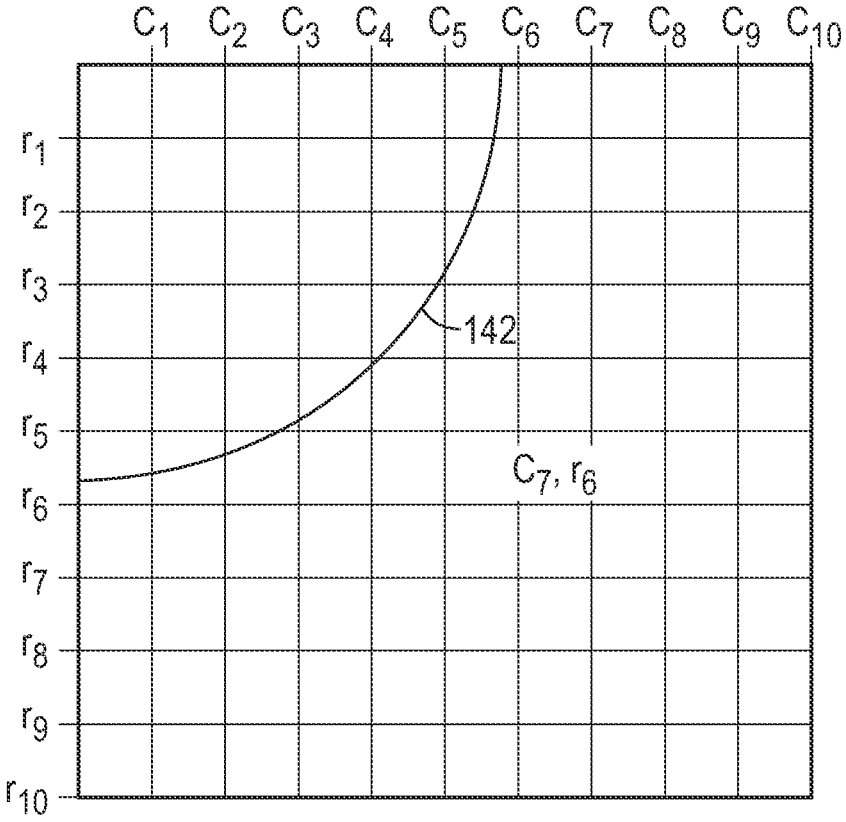
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
r1
r2
r3
r4
r5
r6
r7
r8
r9
r10
142
C7, r6

FIG. 6

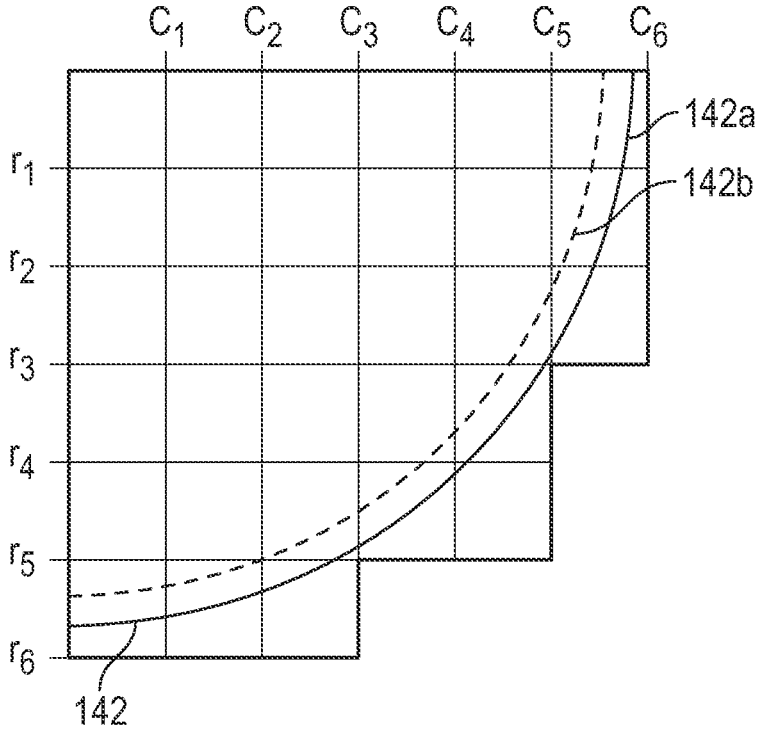
C1
C2
C3
C4
C5
C6
r1
r2
r3
r4
r5
r6
142a
142b
142

FIG. 7

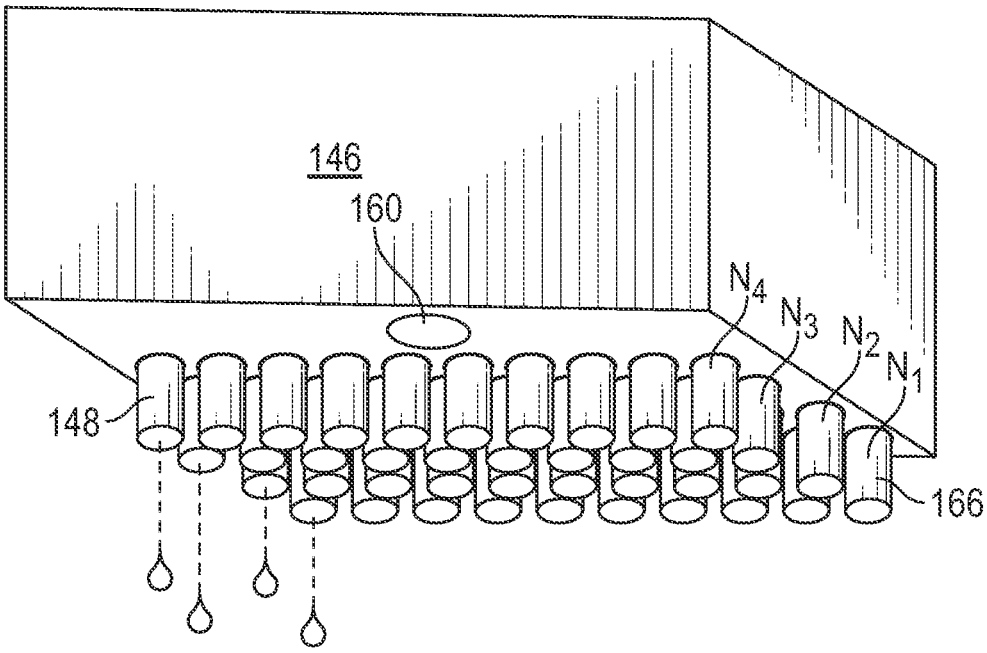
146
160
N_4
N_3
N_2
N_1
148
166

FIG. 8

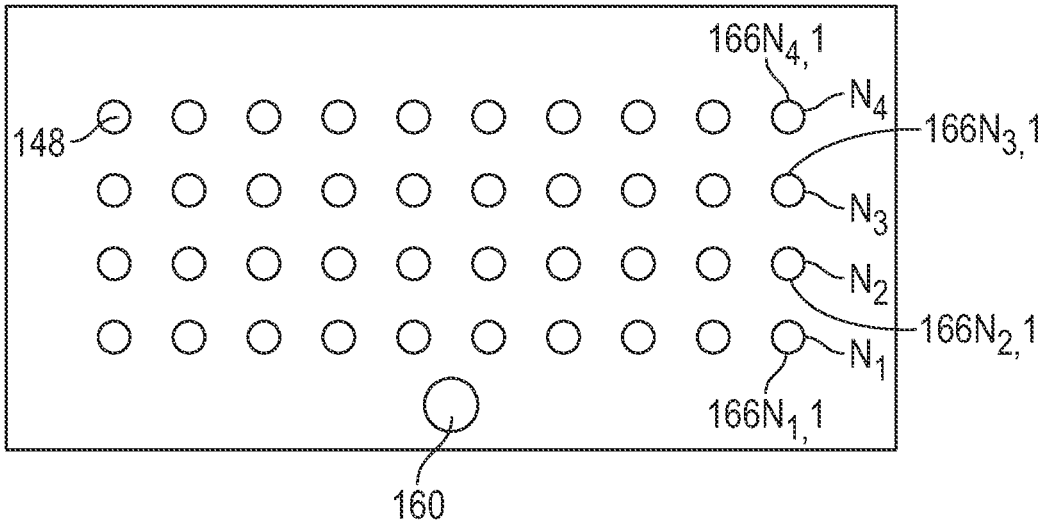
$166N_4,1$
N_4
148
$166N_3,1$
N_3
N_2
$166N_2,1$
N_1
$166N_1,1$
160

FIG. 9

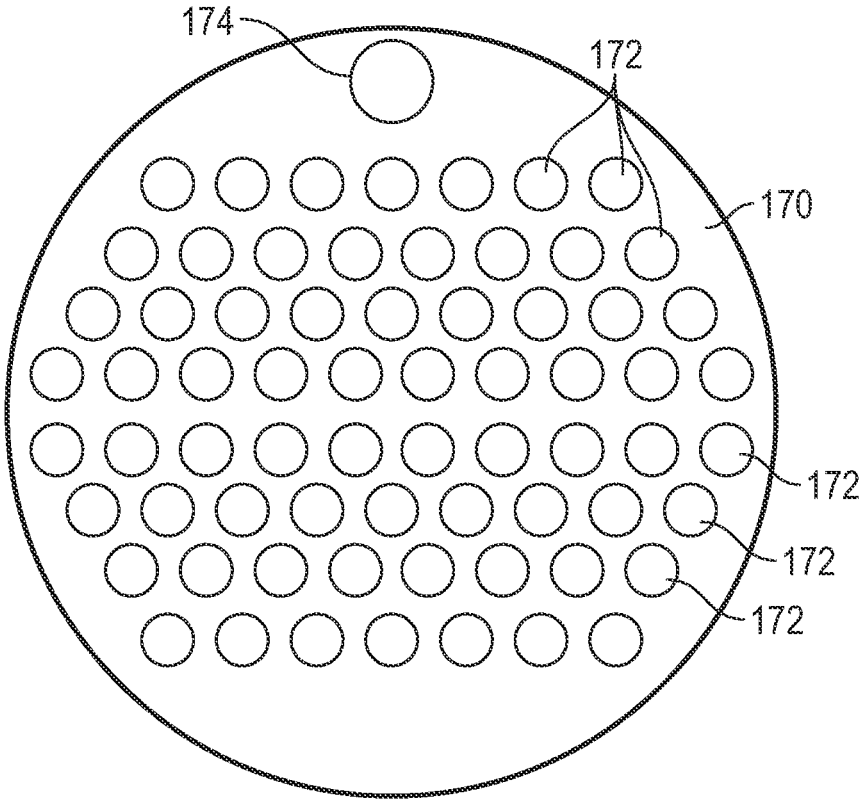

FIG. 10

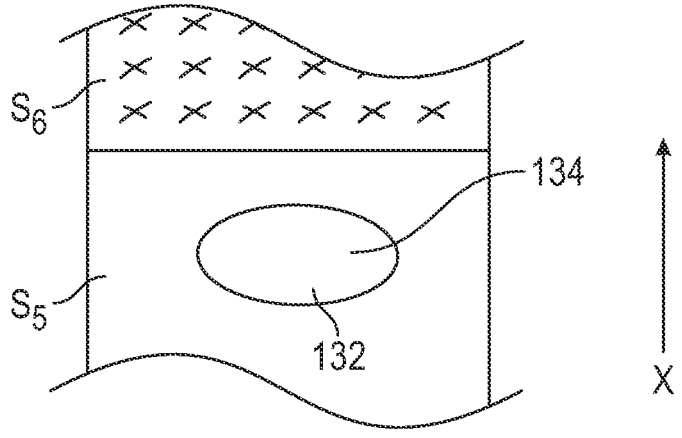

FIG. 11

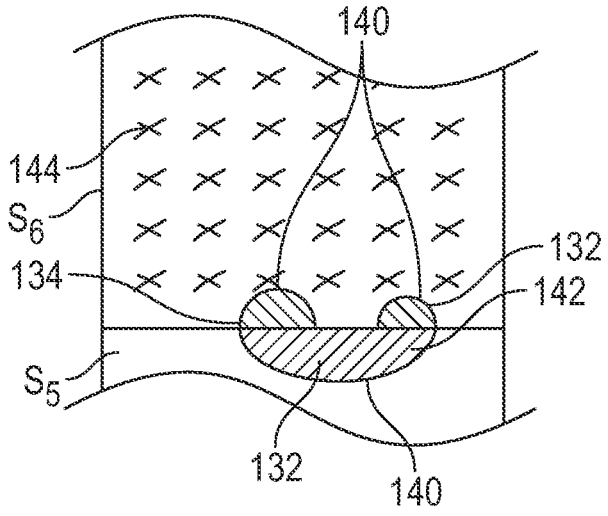

FIG. 12

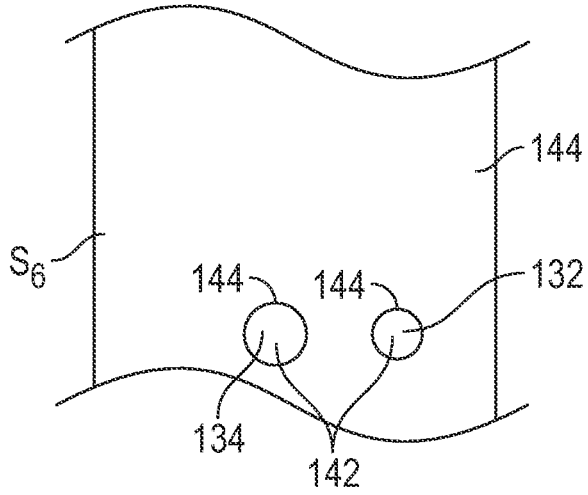

FIG. 13

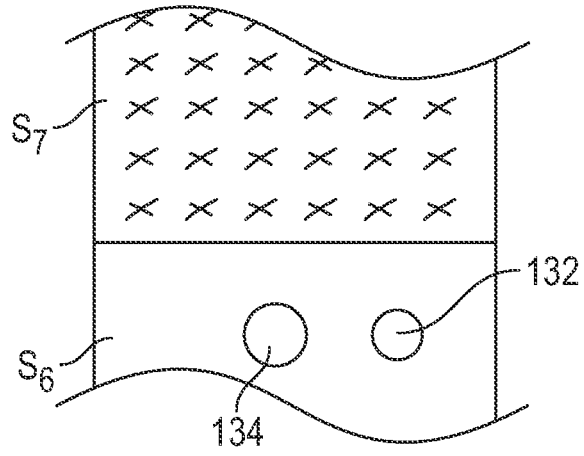

FIG. 14

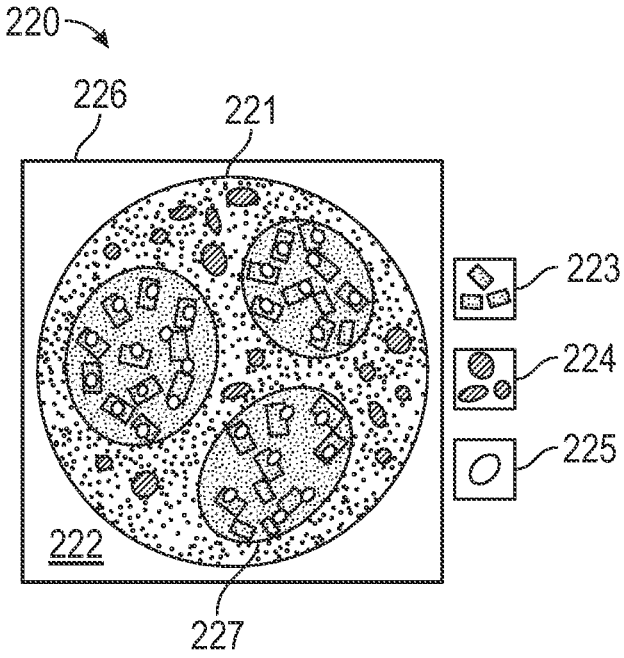

FIG. 15A

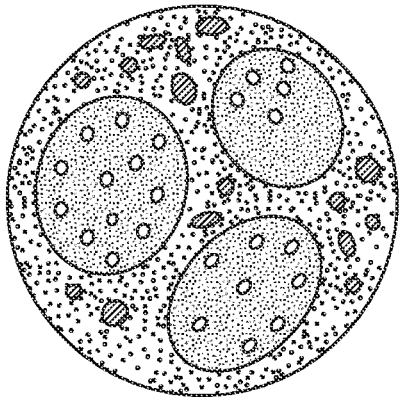

FIG. 15B

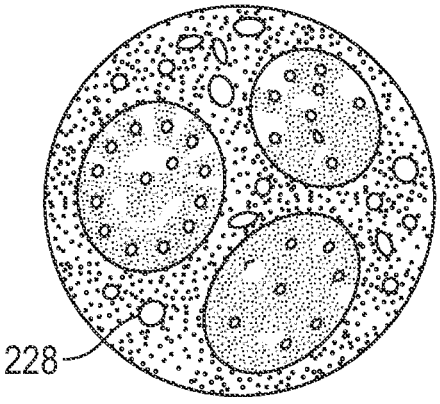

FIG. 15C

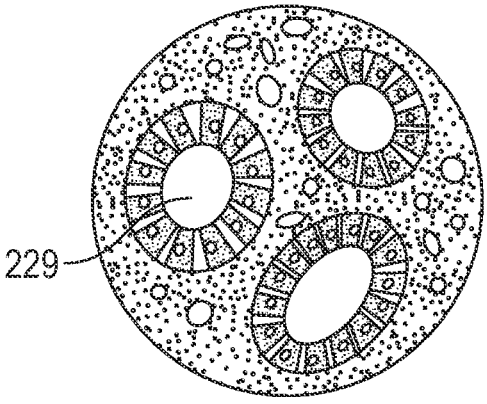

FIG. 15D

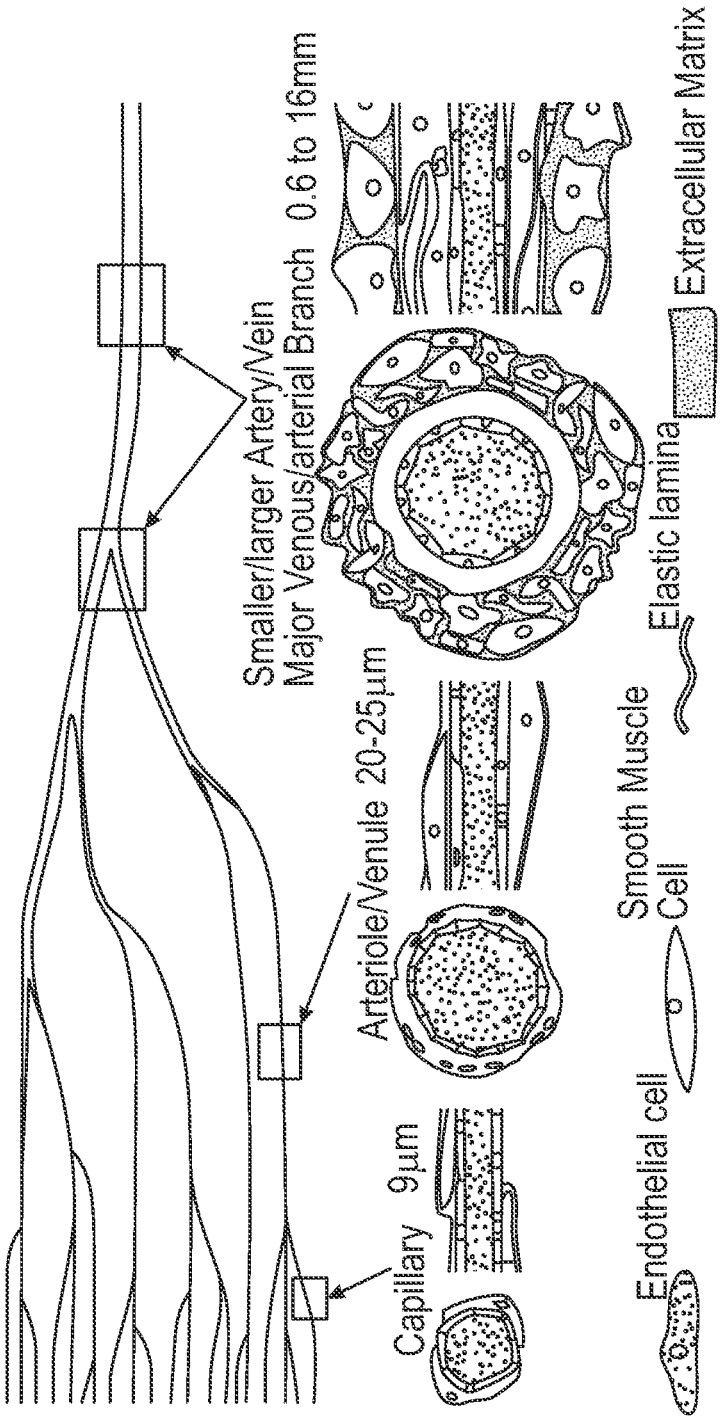
Smaller/larger Artery/Vein
Major Venous/arterial Branch 0.6 to 16mm
Arteriole/Venule 20-25µm
Capillary 9µm
Extracellular Matrix
Elastic lamina
Basement Membrane
Smooth Muscle Cell
Fibroblast
Endothelial cell
Pericyte

FIG. 16

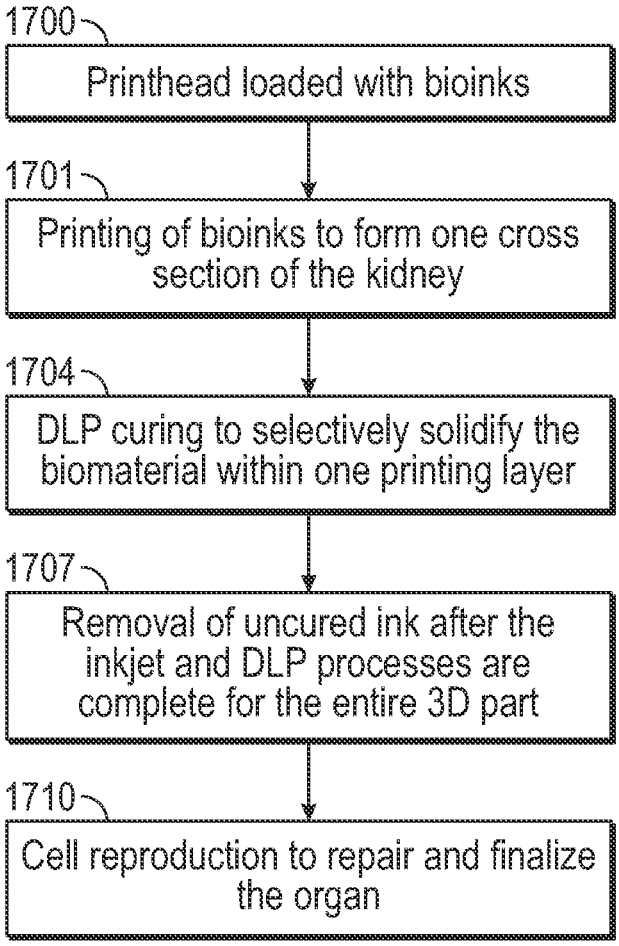
1700
Printhead loaded with bioinks
1701
Printing of bioinks to form one cross section of the kidney
1704
DLP curing to selectively solidify the biomaterial within one printing layer
1707
Removal of uncured ink after the inkjet and DLP processes are complete for the entire 3D part
1710
Cell reproduction to repair and finalize the organ

FIG. 17

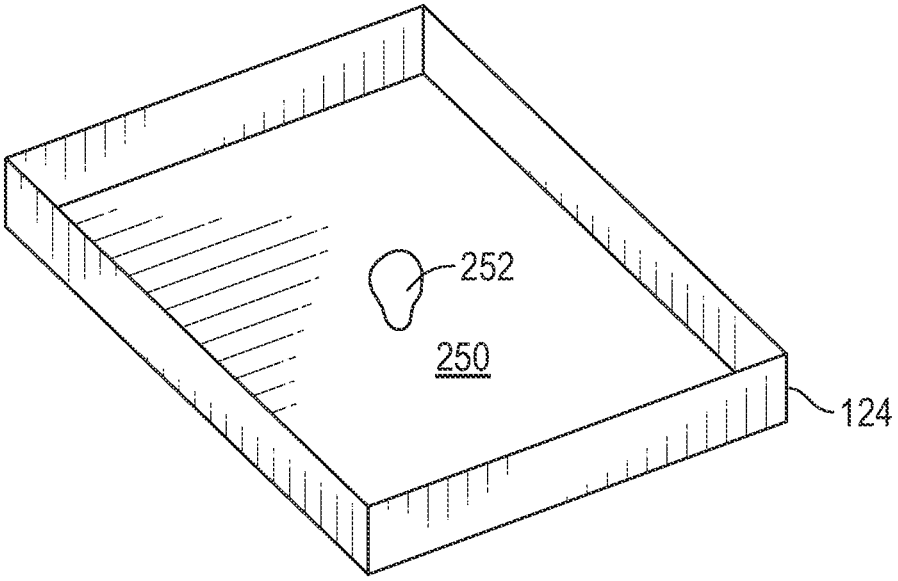

FIG. 18A

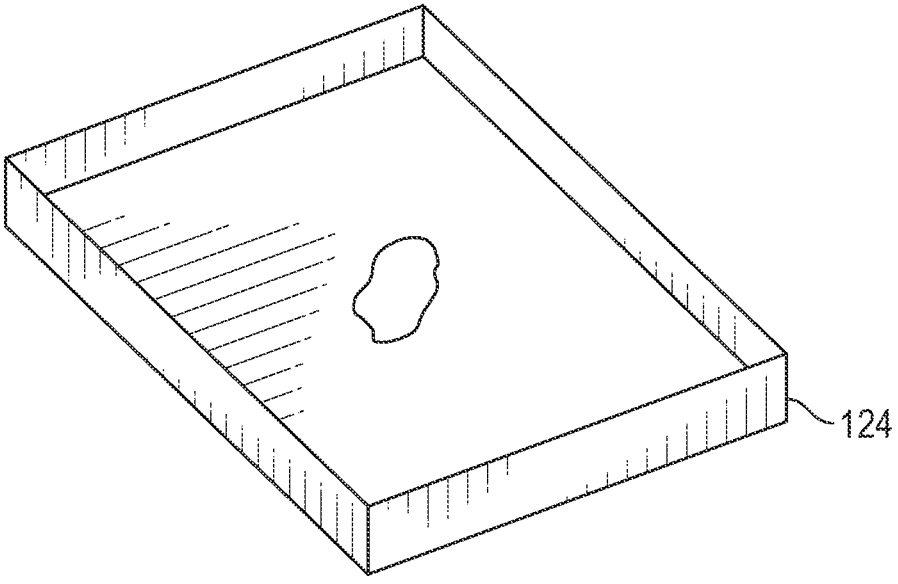

FIG. 18B

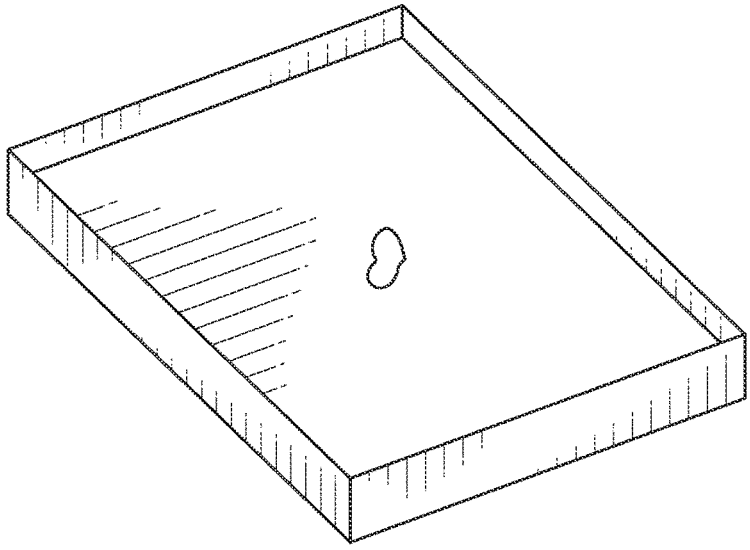

FIG. 18C

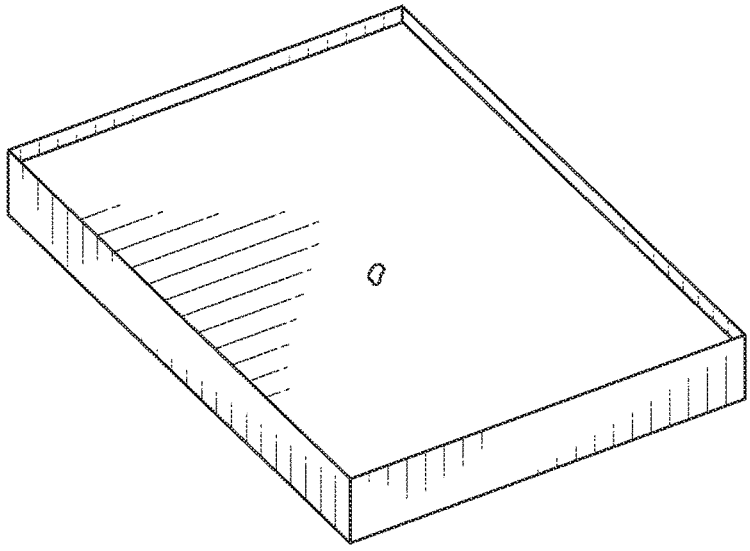

FIG. 18D

HYBRID PRINTING PLATFORM FOR 3D BIOPRINTING OF LIVE ORGANS

BACKGROUND

Field

[0001] A 3D bio-printing scheme that simultaneously allows for micrometer (micron) scale structural resolution and material heterogeneity in a 3D printed object. More particularly, the present disclosure relates to a unique hybrid 3D printing scheme which combines the high-resolution of digital curing technology with the multi-material deposition capability of an inkjet type printer into one platform.

Description of the Related Art

[0002] Objects desired be manufactured using 3D printing techniques, for example human organs, consist of tissue architectures with different material compositions and physical dimensions having material feature sizes as small as micrometer or micron sizes. There are 3D printing technologies such as stereolithography, also known as "SLA" and digital lithographic printing, also known as "DLP" that can provide high resolution, i.e., small features sizes, of printed structures from a light sensitive material layer of a single composition. Inkjet and fused deposition manufacturing, also knowns as "FDM" technologies allow multi-material processing capabilities, but cannot meet the small feature resolution requirements of certain resulting structure created by 3D printing using these technologies. However, none of these existing solutions can meet both requirements at the same time, i, e., both high resolution printing of small features in the micrometer (micron) scale or size of feature, and individual printed layers of slices having material heterogeneity, or multiple different materials at the same level or slice of the printed material. The present invention proposes a new 3D printing scheme to overcome this challenge. The new 3D printing scheme enables micrometer-scale structural resolution of printed structure, and material heterogeneity, at the same time.

SUMMARY

[0003] In a first aspect, a method of forming an artificial organoid having bioactive cells includes dispensing droplets of an electromagnetic energy curable bioink onto a surface to form a plurality of layers of the organoid in liquid form, wherein the droplets of bioink contain bioactive cell types therein of a structure to be formed in the organoid, and each droplet form a layer of bioink on the surface which is larger than a minimum feature size of a structure to be formed by curing the curable bioink, and directing electromagnetic energy capable of curing the bioink having the bioactive cells into the layer of bioink, the electromagnetic energy having a beam width intersecting the bioink which is at least as small as the smallest feature of a structure to be formed in the organoid.

[0004] In an additional aspect, an apparatus useful for forming an artificial organoid having bioactive cells includes a liquid dispensing device capable of dispensing droplets of an electromagnetic energy curable bioink onto a surface to form a slice of the organoid in liquid form, wherein the droplets of bioink contain bioactive cell types therein of a structure to be formed in the organoid, and a droplet forms an area larger than the smallest feature of the structure to be formed in the organoid, and an electromagnetic beam directing device configured to direct electromagnetic energy capable of curing the bioink with the bioactive cells, the electromagnetic energy having a beam area intersecting the bioink which is smaller in width than the smallest feature of a structure to be formed in the organoid.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] So that the manner in which the above recited features of the present disclosure can be understood in detail, a more particular description of the disclosure, briefly summarized above, may be had by reference to embodiments, some of which are illustrated in the appended drawings. It is to be noted, however, that the appended drawings illustrate only exemplary embodiments and are therefore not to be considered limiting of its scope, may admit to other equally effective embodiments.

[0006] FIG. **1** is an isometric view of an organ printer capable of dispensing an electromagnetic energy curable material onto carrier mounted on a moveable stage and of curing that material.

[0007] FIG. **2** is a schematic view of a substrate carrier tray or base on which a 3D bioprinted organ can be formed.

[0008] FIG. **2**A is an enlarged view of a corner of a substrate carrier tray or base on which a 3D bioprinted organ can be formed of FIG. **2**, within the dashed outline circle of FIG. **2**.

[0009] FIG. **3** is a schematic view of a branched lumen.

[0010] FIG. **4** is a sectional view of the branched lumen of FIG. **3**.

[0011] FIG. **5** is an isometric view three slices or sublayers of a branched lumen of FIG. **3**.

[0012] FIG. **6** is a plan view of a portion of a theoretical grid useful for bioink printed on a substrate.

[0013] FIG. **7** is a plan view of a portion of FIG. **6**.

[0014] FIG. **8** is an isometric view of a pint head useful to dispense bioink.

[0015] FIG. **9** is a plan view of the underside of the print head of FIG. **8**.

[0016] FIG. **10** is a plan view of an underside of the output head of the digital lithographic printer of FIG. **1**.

[0017] FIG. **11** is a plan view of a bioink slice being printed over a previously printed and cured bioink slice.

[0018] FIG. **12** is a plan view of the bioink slice being printed over a previously printed and cured bioink slice, showing a feature in the slice being printed being formed as a continuation of a feature in the previously printed and cured slice.

[0019] FIG. **13** is a plan view of the slice being printed in FIG. **12**, now in a cured state.

[0020] FIG. **14** is a plan view of a slice being printed over the printed and cured slice of FIG. **13**.

[0021] FIG. **15**A is a sectional view of a small portion of an artificial organoid being fabricated, and containing a plurality of bioinks deposited on a carrier.

[0022] FIG. **15**B is a sectional view of the of FIG. **15**A small portion of an artificial organoid being fabricated, and containing a plurality of bioinks having at least a portion thereof in a cured state.

[0023] FIG. **15**C is a sectional view of the organoid of FIG. **15**B after being washed to remove uncured bioink therefrom.

[0024] FIG. **15**D is a sectional view of the organoid of FIG. **15**C after being matured or repaired into functional domains.

[0025] FIG. **16** is a schematic view of the types of structures a 3D bioprinting device may manufacture.

[0026] FIG. **17** is a flowchart showing the acts in the manufacture on the bioprinted organ in FIG. **15**D.

[0027] FIGS. **18**A to **18**D are isometric schematic views of an organoid being fabricated in a tray, showing a sacrificial material surrounding, and supporting, the volume of the organoid.

[0028] To facilitate understanding, identical reference numerals have been used, where possible, to designate identical elements that are common to the figures. It is contemplated that elements and features of one embodiment may be beneficially incorporated in other embodiments without further recitation.

DETAILED DESCRIPTION

[0029] Referring initially to FIG. **1**, an inkjet type printer **112** configured to dispense an electromagnetic energy curable material, here a bioink containing living or bioactive cells and a material curable by directing electromagnetic energy therein, onto carrier **124** mounted on a moveable stage **104**, and to cure that material, is shown. Here, the dispense and cure station **100** generally includes a base **102**, a stage **104** moveable in the X-Y plane of Figure X, an upright support **106**, a dispense station **108** and curing station **110** operatively connected to the upright support **106**. In this configuration, the dispense station **108** and the cure station **100** are controllably moveably coupled to the upright support **106** to move in the Z direction of FIG. **1**, but are generally fixed against movement in the X and Y direction by their connection to the upright support **106**. In the construct of Figure X, the dispense station **108** is configured as an inkjet type printer **112** capable of dispensing droplets of material capable of being cured by electromagnetic energy, and the curing station is a what is commonly known as a digital lithographic printer **114**, configured to output one or more wavelength of electromagnetic energy which may be in the range of visible light, as one or more beams of such electromagnetic energy having a beam spot diameter resolution, at the surface of a material dispensed onto a carrier **124** on the moveable stage **104** on the order of **10** p diameter or less, and the inkjet type printer **112** is capable of dispensing droplets of bioink on the order of 50 to 500μ in diameter onto the carrier **124** surface, or a layer of material previously deposited thereon. Thus, by appropriate movement of the stage **104**, and thus a carrier **124** positioned thereon, droplets of a desired biomaterial as a bioink in a liquid phase are dropped onto appropriate locations on the carrier, or onto locations on a previously formed layer of cured, uncured, or cured and uncured droplets, and as the stage **104** indexes in the X direction in the direction of the digital lithographic printer **114**, to enable one or more beams of electromagnetic energy emitted therefrom to reach the dispensed liquid material layer and selectively cure it into a solid form. Here, as will be described further herein, the moveable stage is moveable in the X and Y directions of FIG. **1**, and as each layer of slice of the material used to form the printed bio-material is dispensed and cured, the dispense station **108** and the cure station **100** are controllably moved away from the movable stage **104** by the thickness of the just cured slice by elevation thereof on the upright support **106**.

[0030] Here, base **102** is a generally rectangular, in plain view, open frame **180** having opposed long walls **182**, **184** facing each other across the width of the frame **180** and extending in the X direction of FIG. **1**, opposed short walls **186**, **188** facing each other across the length of the frame **180** and extending in the Y direction of FIG. **1**, and connecting at their opposed ends, to opposed ends of the long walls **182**, **184**, and a plurality of stretchers **190** in the Y direction and extending between, and connected at their opposed ends, to the opposed long side walls **182**, **184** at locations between the opposed short walls **186**, **188**. A guide rail **192** is provided to extend between and is connected at its opposed ends to the opposed short walls **186**, **188**, and it includes a bulbous, in cross section, upper a guide section **194**. A support rail **196** likewise extends between and is connected at its opposed ends to the opposed short walls **186**, **188**, and at the opposed ends thereof are provided opposed first and second end support bosses **200**, **202**, through which extends a lead screw **204**. A drive motor **206**, for example a servo motor or stepper motor, is connected to an end of the lead screw **204** extending through the second end support boss **202**, and is also connected to a motor support bracket **207** which is connected to the support rail **196** to hold the motor **206** stationary while an output shaft thereof, extending through the motor support bracket and connected to the one end of the lead screw **204**, rotates. Lead screw **204** and guide rail **192** both extend in the X direction of FIG. **1**, and parallel to one another. They together form a support for the moveable stage **104** to maintain the stage support surface **116** level, in other words in a plane which is perpendicular to the local direction of gravitation force.

[0031] Moveable stage **104** here is coupled to the upper guide section **194** of the guide rail **192** to be freely slidable, in the X direction of FIG. **1**, thereon, and also receive the lead screw **204** therethrough. Here, moveable stage includes a first support portion **208**, from the lower surface of which extends two support blocks **210**, **212**, each of which includes a lower surface having a reverse bulbous contour thereon which is contoured to receive the upper surface of the bulbous contour of the guide section **194** of guide rail **192** thereagainst, and at least one extending support **216** extending below the first support portion, and including a threaded lead screw opening **218** extending therethrough through which the lead screw extends, the threads on the outer surface of the lead screw **204** received and threaded into the threads in the threaded lead screw opening **218**. As the lead screw **204** and guide rail **192** are spaced from one another in the Y direction, and extend in the X direction and parallel to one another in the light direction of each, the first support portion **208** of the moveable stage is supported at one side thereof in the Y direction by the support blocks **212**, **214** slidably engaged against the guide section **194** of the guide rail **192**, and at the other side thereof by the lead screw **204** extending through the threaded lead screw opening **218** in the extending support **216**. By rotating the lead screw in a first direction (clockwise or counterclockwise when looking at the exposed end of the motor **206** from the X direction, the first support portion **208** moves away from the motor **206** because the rotation of the threads on the lead screw **204** cause the extending support to process therealong in that same direction. Reversing the rotation direction of the lead screw **204**, the first support portion will move in the opposite X direction, i.e., toward the motor **206**. By selecting the pitch of the mating threads on the exterior surface of the lead

screw **204** and mating threads in the in the threaded lead screw opening **218** of the extending support, the movement in the X direction of the first support portion **208** in the X direction can be appropriately selected to meet the desired requirements for movement of the moveable stage **104** desirable for the printing application. For example, micrometer movements in the X direction per 360° rotation of the lead screw **204** are contemplated, and as the lead screw rotation can be controlled in the range of individual degrees on rotation thereof, even smaller controlled X direction movements of the moveable stage **104** are possible herewith.

[0032] As the moveable stage **104** is controllably moved in the X direction, to allow the entire upper surface thereof to be exposable to one of the bioink droplet dispensers of the dispense station **108**, the moveable stage needs to also move in the Y direction of FIG. **1**. Thus, the moveable stage **104** further includes at least one linear driven portion **232**, for example a rack or a linear magnet structure, and at least one linear drive portion **230**, for example a pinion gear drive to drive a pinion gear to move the rack with respect thereto, or a magnetic motor to move the linear magnet with respect thereto, to move the support portion **208** in the Y direction. Thus, by controlling the X and Y position of the support portion **208**, and location on the stage support surface **116** can be positioned under any of one or more bioink dispensers in the dispense station **108**.

[0033] To form a bioprinted member, a carrier **124**, having a carrier base **118** and surrounding walls(s) **120** together surrounding a bioink accessible open top receiving volume **122** of the carrier **124**, is provided to be located on the stage support surface **116** and positioned to receive liquid droplets of biomaterial from the dispense station **108** and move to position the resulting layer or slice of liquid material thereon into a position under the digital lithographic printer **114**, without meaningful change in position of the liquid layer of biomaterial on the carrier **124**, to receive electromagnetic radiation from the digital lithographic printer **114** to cure at least portions of the liquid material layer thereon into a solid form. Thus, the carrier **124** is mounted on the stage support surface **116** of the moveable stage **104**, and the moveable stage **104** is scanned in the X and Y directions to position discrete areas of the interior surface of the carrier base **118** under an inkjet dispense **112** to receive droplets of the biomaterial thereon in discrete desired locations thereon, and then move the dispensed liquid, in substantially the same location on the carrier **124**, or a layer of cured liquid already thereon, as it was dispensed to, to a location under the digital lithographic printer **114** to receive an appropriate dosage, as a function of exposure time and intensity, of electromagnetic energy capable of curing the liquid to a solid form.

[0034] Droplets of bioink to form a layer of bioink, and droplets of a sacrificial material, which can be cured into a solid form using the electromagnetic energy of the digital lithographic printer **114**, are dispensed onto the carrier to form discrete layers or slices of the artificial organoid being built. Each layer has a specific thickness. To ensure process uniformity, the distance between the output optics of the digital lithographic printer **114** and the surface of the layer or slice on the carrier **124** to be cured, or the distance from the opening in a bioink dispenser and the surface of a layer or slice, needs to remain constant. Thus, the dispense station **108** and digital lithographic printer **114** are coupled to a support surface **116**, and a riser lead screw **242** extending through a threaded opening (not shown) in the support plate **240** is rotationally supported on upright support **106**, and a riser motor **244** is coupled thereto to cause rotation of the riser lead screw **242** to enable raising and lowering of the support plate **240**, and thus the digital lithographic printer **114** and the surface of the layer or slice on the carrier **124** and the dispense station **108** with respect to the surface of a layer or slice of material on the carrier, or the carrier base **118** of the carrier **124** itself.

[0035] During the formation of the bioprinted matter herein, a three-dimensional matrix, composed of discrete layers or slices of different curable liquids which are dispensed from the inkjet dispense printer **112**, is formed and selectively cured, wherein a plurality of sublayers of the final bioprinted matter, for example a mammalian kidney, are formed, one over the other, until the entire kidney is printed on the carrier **124**. As the biomatter, here for example an artificial organoid such as an artificial kidney, is composed of a plurality of individual subelements, for example blood vessels, ureter tissue, and other biologically active elements, these vessels, tissues, and other structures will often extend through several of the sublayers or slices of initially, as dispensed, uncured, and then cured, liquid material dispensed onto, and cured sequentially on, the carrier **124**. The organoid being formed, here a kidney, is formed pursuant to a design, in which the individual elements of the kidney, and their interaction with one another, are taken into consideration, and this design is converted into a printing file and a curing file, which represents a sequence of printable slices of the kidney having the thickness of a sub layer or slice thereof, the sublayer or slice having a thickness which is both formable using the inkjet type printer and which inherently possesses sufficient locational definition and stability of the bioink while moved on the carrier **124** from the location where the liquid is received to the location where the liquid is cured with the electromagnetic energy available from the digital lithographic device. Additionally, each printed and subsequently cured slice or sub-layer has to be properly aligned to the slices or sublayers thereadjacent, i.e., immediately above or immediately below, to enable features that extend between slides to be contiguously formed across or through the multiple slices or sublayers.

[0036] To aid in this alignment, carrier **124**, here shown in FIG. **2**, includes a rectangular carrier base **118** and four walls **120**, each extending upwardly from a side of the carrier base **118**, and at least one reference mark **126**, here in the shape of an X, at the intersection location **130** of the upper walls **128** of adjacent walls **120** as shown in FIG. **2**A, an enlarged view of the corner of the tray of FIG. **2**. As will be described herein, the carrier **124** may include a plurality of such reference marks **126**, for example at least two such marks at different intersections **130** of the upper walls **128** thereof, and the inkjet type printer **112** and the digital lithographic printer **114** each have a detection system including a visioning system, such as a camera, CCD, or other such imaging device, that is capable of locating the reference marks to determine the relevant location of the carrier, and thus locate, for purposes of dispensing droplets or curing a material thereon, any location on the upper surface of the carrier base **118** thereof, with respect to the operative elements of the inkjet type printer **112**, the digital lithographic printer **114**, and the moveable stage **104**. Thus, the inkjet type printer **112** and digital lithographic printer **114** can direct the droplets of biomaterial, and the electromag-

netic energy, respectively, to controllably selected locations of the carrier **124** and for the digital lithography printer, previously dispensed liquid, to sequentially create individual slices of the biomaterial with the internal components and features thereof in the proper alignment for the resulting designed biomatter.

[0037] Referring now to FIGS. **8** to **10**, a schematic views of an exemplary printhead of the inkjet printer **112**, and the optical output array of the digital lithographic printer **114**, are shown. To locate the ink of, i.e., print the organoid, as the carrier **124** is moving to the left in FIG. **1**, optics in the inkjet type printer **112**, here through a lens **160** on a printhead array **162** thereof, obtain an image of the reference marks on **126** on the carrier **124** on the ingress end thereof. A controller **164** is operatively connected to the inkjet head to selectively enable individual ones of a plurality of nozzles, here rows of Nozzles N**1** to N**4**, where each nozzle **166** in a row N**1** to N**4** is coupled to the same source of liquid curable material, but the liquid curable material is different from nozzle row to nozzle row, to open to dispense a liquid bioink therefrom. Thus, for example, nozzle row N**1** includes ten nozzles $\mathbf{166}_{n1,\ 1}$ to $\mathbf{166}_{n1,\ 10}$, nozzle row N**1** includes ten nozzles $\mathbf{166}_{n1,\ 1}$ to $\mathbf{166}_{n1,\ 10}$, nozzle row N**3** includes ten nozzles $\mathbf{166}_{n3,\ 1}$ to $\mathbf{166}_{n3,\ 10}$, and nozzle row N**4** includes ten nozzles $\mathbf{166}_{4,\ 1}$ to $\mathbf{166}_{n4,\ 10}$. The pitch, or spacing, between adjacent ones of the nozzles **166** in any row N_1 to N_4 are approximately equal to, or smaller than, the area over which the liquid dispensed therefrom will spread out when it contacts an underlying surface, for example, the area of a grid locale such as locale c_7, r_6 of FIG. **6**. For example here, assume that the nozzles of row N_1 dispenses a first bioink **140** containing lumen wall cells, the nozzles in row N_2 dispense a sacrificial material, and those in row and row N_3 dispense a third bioink **145** having a different cell than the lumen wall cell therein.

[0038] The digital lithographic printer **114** includes, as shown schematically in FIG. **10** which is a view of the stage facing side of a litho head **170** of the device, a plurality of electromagnetic energy beam outlets **172** and a beam synching optics pickup **174**, connected to a camera (not shown) within the litho head **170**. Controller **164** is likewise coupled to the camera and to the individual beam optics of the energy beam outlet **172**, to selectively cause the energy beam outlets **172** to emit, or not emit, electromagnetic radiation, based upon which area of the slice of bioink passing thereunder intersects with the output of a particular energy beam. Thus, as each slice of liquid dispensed from the inkjet type printer **112** moves under the litho head **170**, the individual electromagnetic energy beam outlets **172** selectively direct beam energy at specific areas of the slice. Here, the area of an electromagnetic energy beam emitted by any of the energy beam outlets **172** is less than the area that an individual droplet of bioink will cover on the base of the carrier **124** or a layer already formed thereon, for example, smaller than grid locale **c7**, **r6** of FIG. **6**. Thus, the resulting cross section of the bioink which is cured by the electromagnetic energy beam emitted by any of the energy beam outlets **172** is less than the area that an individual droplet of bioink has covered on the base of the carrier **124** or a layer already formed thereon, and thus a finer resolution, i.e., small sized feature, can be formed which is not limited in smallness of size by the size of the bioink droplet.

[0039] As a printing example, FIGS. **3** to **5** show an exemplary feature of a biomatter, here a branched vasculature or branched lumen **132** having a main lumen **134** portion, and a branch lumen **136** extending therefrom. In FIG. **3**, the branched lumen is shown in an isometric view, whereas in FIG. **4** it is shown at section **4-4** of FIG. **3**, for ease of demonstrating the slices or sublayers of bioink used to form the branched lumen **132**. As shown in FIG. **4**, the branched lumen **132** extends through multiple slices or sublayers of bio ink (and resultantly cured bioink), here slices S_1 to S_7 of a bioink to form the artificial organoid to be fabricated, each slice having a thickness which is both capable of being formed in liquid form on the carrier **124** or on a previously formed slice formed on the carrier **124**, and being effectively cured using electromagnetic energy of the digital lithography printer **114**. Here, only the branched lumen **132** of the artificial organoid being fabricated is shown for ease of illustration. However, it should be appreciated that each slice or sublayer of bioink, and thus of the resulting fabricated artificial organoid, is likely to include multiple portions of lumens and other organoid structural materials, which are aligned from slice to slice or sub-layer to sub-layer to fully fabricate the artificial organoid.

[0040] To print the branched lumen **132** of FIG. **3**, for example the upper three slices thereof S_5 to S_7, different bioinks containing different biologically active or inactive materials must be employed. A first bioink **140** contains the bioactive material, i.e., bioactive lumen wall cells, from which the lumen wall is formed, here the lumen wall material. A second bioink **144** is also employed, which may also include biological cellular material used to form other features of the biomatter, filler material such as a sacrificial material, or combinations thereof. Once slice S_5 is formed in proper alignment with the underlying slice S_4, the stage support surface **116** is moved to the far right of the base **102** of FIG. **1**, to a fixed potion, in the Y direction, which is a reference position for the stage support surface **116**. Here, carrier **124** is mounted to the stage support surface **116**. The carrier **124** here has opposed ends, for reference an ingress end **152** which is the end thereof which, when the stage support surface **116** initially moves to the left of FIG. **1**, comes into place below the inkjet type printer **112**, and an egress end **154**, which is the last part of the carrier **124** to pass under the inkjet type apparatus **112** as the liquid forming a slice is deposited thereon or on a layer previously formed thereon.

[0041] To print this branched lumen, and the material thereabout, a printing file is created based upon the design architecture of the artificial organoid, which creates individual ordered sub-layers or slices of the artificial organoid, and also identifies, in an X-Y plane of the slices or sublayers, for example the S**5** to S**7** of FIG. **5**, individual printable locations on the slice as shown in FIG. **6**. In FIG. **6**, a plurality of rows **r1**-**r10**, and columns **c1** to **c10** are shown, and each represents the outline, and area, of the minimum resolution or size of a droplet of bioink that can be formed on the carrier **124** or a slice or sub-layer previously formed on the sublayer. The interstices of the columns and rows are shown as individual squares, each having sides of a length l. As droplets of bioink are dispensed onto the uppermost surface in the carrier **124**, they will spread until contacting another drop, and their individual outlies will not necessarily be square. However, at the scale of the size of the droplets, a square is an acceptable mathematical compromise useful in the printing system hereof. For example, l may be on the order of 10 microns in length where the minimum droplet

size will spread to a surface area of 10 microns across in the X-Y plane of the surface onto which it was dropped, such that each square, for example square r**4**, c**6** is 100 microns in area in an X-Y plane.

[0042] For printing of the bioinks, here first and second bioinks **140**, **144** to form the branched lumen **132** structure of FIGS. **3** to **5**, each slice of the organoid is mathematically converted into a print file containing the bioink locales in column and row form for dispensing thereof into a grid pattern such as that shown in FIG. **6**, except extending over the maximum X-Y dimensions of the widest portions of the artificial organoid being fabricated. Here, the grid of FIG. **6** is the small part of the slice S**5** of FIG. **5** shown in dashed outline. Here, the outer surface **142** of the branched lumen at slice S**5** is shown as an arc extending across the grid of FIG. **6**, and in each square locale (row-column intersection) within the circumference of the outer wall, the first bioink is deposited by the inkjet printer **112**, and in region outwardly thereof, the second bioink is deposited. Where the outer wall **142** passes through a grid locale box, the first bioink is deposited, as shown in FIG. **7**, where only those grid locales having the first bioink therein are shown, the remaining grid locales of FIG. **6** containing the second bioink. Thus, for the branched lumen **132** of slice S**5**, the entire volume thereof circumscribed or surrounded by furthest extent or outer surface **142***a* of the contemplated outer wall **142** thereof will receive the bioink from which the outer wall is formed, although in the finished branched lumen the interior of the lumen inwardly of the outer wall **142** need remain open for the eventual flow of a liquid, blood, therethrough.

[0043] To locate the ink of, i.e., print the branched lumen **132** and surrounding material, as the carrier is moving to the left in FIG. **1**, optics in the inkjet type printer **112** obtain an image of the carrier **124** and reference marks **126** on the egress end thereof through a lens **160** on a print head array **162** thereof. A controller **164** of the inkjet type printer is operatively connected to a plurality of nozzles, here rows of Nozzles N**1** to N**4**, where each nozzle **166** in a row N**1** to N**4** is coupled to the same source of liquid curable material, but the liquid curable material is different from nozzle row to nozzle row. Thus, for example, nozzle row N**1** includes ten nozzles $\mathbf{166}_{n1,\ 1}$ to $\mathbf{166}_{n1,\ 10}$, nozzle row N**1** includes ten nozzles $\mathbf{166}_{n1,\ 1}$ to $\mathbf{166}_{n1,\ 10}$, nozzle row N**3** includes ten nozzles $\mathbf{166}_{n3,\ 1}$ to $\mathbf{166}_{n3,\ 10}$, and nozzle row N**4** includes ten nozzles $\mathbf{166}_{4,\ 1}$ to $\mathbf{166}_{n4,\ 10}$. the pitch, or spacing, between adjacent ones of the nozzles **166** in any row N_1 to N_4 are approximately equal to, or smaller than, the area over which the liquid dispensed therefrom will spread out when it contacts an underlying surface. For example here, assume that the nozzles or row N_1 dispenses first bioink containing lumen wall cells, row N_2 dispenses a second bioink **144** containing a different bioactive cell therein, and row N_3 dispenses the third material.

[0044] Referring to FIGS. **11** to **14**, the printing of an exemplary slice S**6** of FIG. **5** over exemplary slice S**5** of FIG. **5** is shown, is shown, wherein the carrier **124** on which the slices S**1** to S**5** are already formed is moving in the X direction from the right to the left in Figure X such that the third material **144** has been dispensed in drops as a portion of slice S**6** over the portion on slice S**5** that does not include any portion of the branched lumen **132** therein. As the carrier **124** moves the slice S**5** further toward the left hand side of Figure X, the location of the branch lumen **132** comes below the printhead array **162**. Note, at slice S**6** the branched lumen **132** branches such that a branch lumen **136** begins branching out from the main lumen **134** portion of branched lumen **132**. Here, slice S**5** includes cured first bioink **140** forming the lumen wall, surrounding a volume of uncured first bioink, and portions of cured and uncured second bioink in the region surrounding the cured first bioink **140**. Thus, when the location of main lumen **134** is encountered, a portion of the liquid (uncured) first bioink **140** is printed over with the second bioink **144**, and the lumen wall material of the first bioink **140** is dispensed in the lumen locations of the branch lumen **136** and main lumen **134**, including area of the to be formed wall and the area circumscribed by the to be formed wall, each of which areas or regions are smaller than the main lumen **134** portion in slice S**5**. As the slice S**5** continues to move, the material of the walls of the main lumen **134** and branch lumen **136** are surrounded in a field of second bioink **144**.

[0045] After the bioink is dispensed, as the moveable stage moves the carrier in the X and Y direction to locate the appropriate nozzle to dispense the appropriate bioink over the grid locale directly therebeneath, portions of the just dispensed bioink will come below the energy beam outlets **172**, which are selectively controlled to direct electromagnetic energy into all, or only a portion of, the bioink at any grid locale of the slice or sub-layer being printed and cured.

[0046] As the stage is passing under the inkjet type printer **112**, it begins to pass under the digital lithographic printer **114**, which includes, as shown in FIG. **10** which is a view of the stage facing side of a litho head **170** of the device, a plurality of electromagnetic energy beam outlets **172** and a beam synching optics pickup **174**, connected to a camera (not shown) within the litho head. As each slice of liquid dispensed from the inkjet type printer **112** moves under the optics head, the individual electromagnetic energy beam outlets **172** direct beam energy at specific areas of the slice. For example, here the beam will define, within the layer of liquid lumen wall material of the first bioink **140**, the actual lumen wall of the desired thickness (width in the X-Y plane of Figure X**5**), which may be less than the corresponding width of the lumen wall material of the first bioink **140** at that location. As shown in FIG. **7**, where the portion of the portion of the slice of FIG. **7** which includes the first bioink **140** thereon, after curing of this material, the lumen outer wall **14***a* and lumen inner wall **14***b* define the radial limits of the cured portion of the first bioink **140**, and that dimension is smaller than the side dimension of any grid locale that could be filled with a single droplet of bioink to the desired slice or sublayer thickness. Thus, although the minimum feature size that can be resolved using the inkjet type printer **112** is not the minimum feature size that can be resolved here, as the electromagnetic energy emanating from the beam outlets **172** can cure a region of the slice on the order of 10μ in diameter, significantly smaller than the possible minimum diameter of the liquid cell wall material **140** on the carrier **124** or a slice previously cured thereon. Thus, the electromagnetic beam selectively cures the liquid materials on the carrier or slice, and the portions of the liquid material which are not cured remain in place so to support the next layer of liquid to form the next slice, but like the sacrificial material, will be washed away from the final organ when the organ is fully printed with the liquid materials.

[0047] Once the entire layer S**6** is printed and selectively cured, the moveable stage **104** returns to the far right of FIG. **1**, and then starts again moving to the left in the X direction

and positioning the slice S**6** relative to the nozzles **166** to dispense the appropriate materials of the lumen wall material of the first bioink **140**, sacrificial material and third material at their appropriate locations to form lumen walls of the main and branch lumens in the slice S**7**.

[0048] As the organoid is being fabricated on the carrier **124**, the size of the organoid in each slice can, and likely will, change. Here, for example, the nozzles in the first three nozzle rows n**1** to n**3** are connected to sources of bioink containing living cells, and the nozzles in each row n**1** to n**3** are dedicated to a single type of bioink. Additionally, the nozzles of the fourth nozzle row are connected to a sacrificial material, i.e., a material which does not contain live cells and which is easily removed from the organoid being fabricated using a biocompatible washing material, such as water. Thus, as shown in FIGS. **18**A to **18**D, as the bioinks of nozzle rows n**1** to n**3**, collectively bioinks **252**, are deposited onto the carrier **124** or a sub layer or slice previously formed thereover, the sacrificial material **250** is dropped, as droplets, from the nozzles in nozzle row **4**, such that the sacrificial material **250** forms a mechanical support layer to support the sides of the slice or sublayer of the bioinks **252**, to help maintain the architectural integrity of the slices of the organoid as they are formed, to thereby help ensure that the use of the alignment system of the apparatus herein will properly align features of subsequent slices or sublayers with respect to the slices or sublayers therebelow.

[0049] Nozzle array **146** here is shown as including forty nozzles **148**, laid out in four rows n**1** to n**4** of ten nozzles each. Each nozzle **148** in a row may, as previously described, be fluidly coupled to a common bioink or other type of curable liquid, or each nozzle **148** may be connected to a discrete type of curable liquid, which may be a bioink having living cells therein. Thus, where an organoid requires, for example, ten cell types to be fabricated, then for example ten sets of four nozzles **148** may configured so that each set dispenses a different curable liquid.

[0050] Controller **164** is provided, which may include a general purpose computer, capable of receiving an instruction set for execution of control of the motors **244**, **206**, digital lithographic printer **114**, and the moveable stage Y direction motion, to selectively open any one or more nozzles simultaneously to dispense curable material therein to a known desired locale with respect to the reference marks **126** on the carrier, and likewise emit curing radiation to desired locations of the carrier relative to those reference marks **126**, to control the dispensed locales of the curable materials, and the curing of that material, to form each slice of the organoid, and align each slice as it is being formed to the slice immediately below it.

[0051] Referring now to FIGS. **15**A-B, a cross sectional view of printed biomatter, for example of a 3D printed kidney is shown. In one embodiment, a portion of a luminal structured functional organoid **220**, in this embodiment a kidney, is printed by the printing device dispense and cure station **100** on the carrier **222** onto which the biomatter is printed. Here, to print a 3D kidney, a structural design of a kidney is converted, using the controller hereof, into individual slices of kidney, including the location of different cell types thereof in each slice, such that individual slices of the kidney can be printed, one over the other, into a complete and functionable organ. Each slice therefore results in an organized biostructure, such as that shown in shown in FIG. **15**D, which is an example of a printable portion of a biomaterial showing the exposed upper layer of the biomaterial which has been formed using 3D bio printing.

[0052] Here, three different kinds of bioinks including a biocompatible electromagnetic energy curable material and one of bioactive kidney cells **223**, bioactive vasculature cells **224** and bioactive extracellular matrix cells **225** are provided and are dispensed and cured using the inkjet type printer **112** of FIG. **1** hereof to form a 3D printed kidney. These bioactive cells **223**, **224** and **225** can form the functional cellular matrix of the resulting 3D printed kidney. To form this structure, the print head dispenses different bioinks each having one of the bioactive kidney cells **223**, bioactive vasculature cells **224** and bioactive extracellular matrix cells **225** therein to print regions of bioink having kidney cell regions of ink, vasculature cell regions of ink, and extracellular matrix cell regions of ink in corresponding generally to the locations of kidney cells **223**, vasculature cells **224** and extracellular matrix cells **225** in each slice of the kidney being printed. The droplet size **221** of the kidney, vasculature, or extracellular matrix cells **223**, **224** and **225** which are dispensed are on the order of 10 um or greater. In other words, the smallest droplet size that can be dispensed onto the carrier **222** is 10 um, but larger droplet sizes can be dispensed on the order of 40-100 um, depending on the size of area of the slice required to be dispensed to that location or region of the slice being printed. An exterior sacrificial fluid **227** is also dispensed in droplets and is located around the exterior of the structure of each slice to serve as a support scaffold to support the outer structure of the kidney or other mammalian tissue as it is being printed.

[0053] Following droplet placement onto the carrier **222**, or onto a previously printed and cured slice, the carrier **222** is moved under the curing station **110** and individual pulses of UV energy are directed, at preplanned intervals, into the just dispensed bioink in order to selectively cure the bioinks in the just dispensed slice. The droplets of bioink from which the kidney cells and extracellular matrix of the kidney are formed are generally of a size which is smaller than the area of the slice in which they are formed, and thus one, and often, multiple droplets of these bioink types are dispensed adjacent to one another to form the regions of the kidney cells and extracellular matrix in each slice. However, in some cases the actual final dimension where the bioactive cells in the bioinks is to remain is smaller than the smallest droplet size, and thus smaller than area of the slice where that bioink is dispensed. This is primarily an issue with the vasculature of the biomatter, here of the kidney being printed, wherein the vasculature wall is typically smaller in width, i.e., the wall thickness of the vessel or lumen from the inner surface to outer surface thereof, than the smallest area on which the bioink is present in the slice. However, here, the individual pulses of electromagnetic energy being emitted from the curing station **110** may be as small as submicron size in cross section, and thus an outer perimeter area of the vasculature cells to form a vasculature wall of bioactive vasculature cells **224** may, where required by the design of the biomatter, be cured with the electromagnetic energy of the curing station to have a cured area of a range below 10 um, for example, 1 um, and leaving liquid within, and in some cases exteriorly of, the resulting cured vasculature walls of each slice. A wavelength range of electromagnetic energy that will cure the polymer in the ink but not meaningfully deteriorate for cells is selected for this method. The cured polymer maintains the cells in place in the slice,

allowing them to grow and replicate in place to form the biome and structure of the biomatter being printed, for example here, a mammalian kidney.

[0054] Following UV curing of the bioinks, areas of uncured bioink **228** remain in place to physically support the bioink of the next slice to be printed or dropped on the just cured slice. Additionally, around each of the slices, outside of the functional volume of the slice of kidney being dispensed, the sacrificial ink is dispensed, and then cured. This cured sacrificial ink is water soluble, and not rigid but help against movement by the walls of the carrier **222**, such that the finished biomatter, here the kidney, becomes nearly encapsulated thereby, but is easily removed by peeling away the finished kidney from the sacrificial material **227**. Additionally, the uncured bioink, and the cured sacrificial material potentially adhering to the outer surface of the finished kidney are **12** are flushed from the completed kidney at a wash station. As the sacrificial material **227** and uncured bioinks are water soluble, washing with water will allow the remaining liquid and cured sacrificial material to be removed without disrupting the cured structures of the biomatter. As the sacrificial fluid is washed out, the printed cured structures containing cells and other biocompatible materials necessary for cells to reorganize into biologically functional organ domains remain in situ. During the washing process gaps will be left in areas between cured portions of the biomatter, as well as shrinking of the sub domains of different cells types occurs and they can detach from surrounding areas, and liquid has been washed away to leave purposefully hollowed out regions or areas within the printed biomatter. The carrier **222** with the finished being printed and cured organ or biomatter is located in a conditioning device and incubated under appropriate conditions for cell growth so that internal gaps in the printed and cured biomatter are matured or repaired and sub domain structures therein are organized. As a result, material boundaries and structural boundaries are decoupled and are not limited to the size of the droplets.

[0055] Referring now to FIG. **17**, a flowchart useful for manufacture of a 3D bioprinted organoid is shown. At Act **1700** the bio-inks are provided to the print head array **162** of FIG. **1**, and each nozzle, or each nozzle row N**1** to N**4** is couples to a different one of the bioinks, or other nozzle to bioink pairings are provided. Each nozzle **166** can have different physical (e.g., construction material, nozzle size, native resolution, viscosity/surface tension range) and process (temperature, meniscus pressure, frequency and waveform) parameters individually optimized for the jetting behavior (drop size, speed and shape, etc.) of each bioink. At Act **1701** the bio-inks are digitally deposited through single or multi pass inkjet printing to form a slice of the organoid. This step defines the gross material boundaries within the layer. Regions for very fine bio-structures (e.g., capillaries) generally require small drop size and high printing resolution, typically drop sizes resulting in areas of bioink of 40 um or less when deposited on the carrier **124** or a slice previously deposited thereon. Whereas areas for larger structures such as extracellular matrix (ECM) materials can be formed with more coarse resolution and larger drops to optimize process throughput.

[0056] At Act **1704**, the fluidic slice of the organoid layer is selectively cured to form high resolution patterns, defining the structural boundaries therein. In this case, contours of the cured area should remain within their material boundary to ensure material homogeneity within the solidified, i.e., cured, structure, and a gap between adjacent two contours can range from a few micrometers to sub-millimeter sizes depending on inkjet resolution, material diffusion coefficients and material purity requirements. Here, for the vasculature of the organoid, a circular, elliptical, or other outline or shell is cured within the bioink containing the vasculature cells **224**, while leaving the inner core thereof uncured. This will allow the cured shells to eventually grow into the blood vessels and capillaries of the kidney. At Act **1707** the uncured inks are removed through by transferring the substrate to a washing station to remove uncured formulations. This will result in gaps between different bio-components therein. At Act **1710** the substrate with the pre organ is placed into an incubator with appropriate conditions for growth, for example in 5% CO_2 at 37° C. The result of this incubation is a luminal structured functional organoid **220**.

[0057] Referring now to FIG. **18**, in FIG. **18***a*, as the initial slice of the organoid is formed, a region of bioinks **252** of a relatively small size is surrounded by a layer of sacrificial material **250** having the same thickness as the slice or sub-layer of the bioinks **252**. As additional slices or sublayers of bioink **252** are formed, the slices or sub-layers have a larger cross section than the slices or sublayers below, and thus the surrounding layers of sacrificial material **250** supports the partial organoid from both the perimeter of the slice being printed, but from below as well, to maintain the architectural integrity of the organoid being fabricated. Then, as the fabrication of the organoid is substantially completed, the area of the slices or sublayers of the bioinks **252** become smaller as shown in FIG. **18**C, but the entire organoid is structurally supported by the sacrificial material, to maintain the architectural integrity thereof. Eventually, as shown in FIG. **18**D, the final slice or sublayer of bioink **252** of the organoid is printed, still surrounded by the sacrificial material. Thus, as a three dimensional organoid is fabricated, portions thereof which must be formed and which extend outwardly from the area of a previously printed slice or sub-layer may be supported, without impacting the functionality of the resulting organoid.

[0058] To facilitate understanding, identical reference numerals have been used, where possible, to designate identical elements that are common to the figures. It is contemplated that elements and features of one embodiment may be beneficially incorporated in other embodiments without further recitation.

What is claimed is:

1. A method of forming an artificial organoid having bioactive cells, comprising:

dispensing droplets of an electromagnetic energy curable bioink onto a surface to form a plurality of layers of the organoid in liquid form, wherein the droplets of bioink contain bioactive cell types therein of a structure to be formed in the organoid, and each droplet form a layer of bioink on the surface which is larger than a minimum feature size of a structure to be formed by curing the curable bioink; and

directing electromagnetic energy capable of curing the bioink having the bioactive cells into the layer of bioink, the electromagnetic energy having a beam width intersecting the bioink which is at least as small as the smallest feature of a structure to be formed in the organoid.

2. The method of claim 1, wherein the bioink is selectively cured using the electromagnetic energy.

3. The method of claim 2, further comprising forming multiple layers, one over the other, to form an organoid after completely forming all of the layers; and

selectively curing all of the layers.

4. The method of claim 2, further comprising forming multiple layers, one over the other, to form an organoids; and

incubating the organoid under appropriate cell growth conditions to mature or repair the organ.

5. The method of claim 1, wherein a bioink droplet forms a bioink layer on the surface as small as 10 um.

6. The method of claim 5, wherein the curing selectively solidifies the biomaterial to a size having a cured dimension less than 10 um in size.

7. The method of claim 1, wherein the surface is a previously formed, and cured layer of bioink.

8. An apparatus useful for forming an artificial organoid having bioactive cells, comprising:

a liquid dispensing device capable of dispensing droplets of an electromagnetic energy curable bioink onto a surface to form a slice of the organoid in liquid form, wherein the droplets of bioink contain bioactive cell types therein of a structure to be formed in the organoid, and a droplet forms an area larger than the smallest feature of the structure to be formed in the organoid; and

an electromagnetic beam directing device configured to direct electromagnetic energy capable of curing the bioink with the bioactive cells, the electromagnetic energy having a beam area intersecting the bioink which is smaller in width than the smallest feature of a structure to be formed in the organoid.

9. The apparatus of claim 8, further comprising a stage mounted on a moveable sub configured to selectively position the surface onto which the droplets are located below the liquid dispensing device.

10. The apparatus of claim 9, wherein the moveable sub is further configured to move the sate under the electromagnetic beam directing device.

11. The apparatus of claim 10, wherein the direction from the surface is positioned on, or in, a carrier, and the carrier is positioned on the stage.

12. The apparatus of claim 10, further comprising a controller operatively coupled to the moveable sub, the electromagnetic beam directing device and the liquid dispensing device.

13. The apparatus of claim 8, wherein the liquid dispensing device includes at least two nozzles selectively openable to release a curable liquid therefrom, wherein the plurality of nozzles include a first nozzle connected to a supply of a first liquid having a first cell type therein, and a second nozzle connected to a supply of a second liquid having a second cell type therein, and the first cell type and the second cell type are different.

14. The apparatus of claim 13, wherein, a droplet of the first liquid forms a layer on the surface which is smaller than a feature to be cured therein, and the second liquid forms a layer on the surface which is larger than a feature to be cured therein.

15. An apparatus for forming a three dimensional object by sequentially dispensing liquid, in droplet form, to form a first liquid layer on a surface, at least partially curing that first layer to form a first feature having a minimum dimension therein, sequentially dispensing liquid, in droplet form, to form a second layer on the first at least partially cured layer, and, at least partially curing that second layer to form a second feature having a minimum dimension therein, comprising:

a liquid dispensing device including at least one selectively openable nozzle connected to a supply of a liquid material, the nozzle capable of dispensing therefrom a droplet of at least a minimum volume, that droplet, when in contact with one of the surface of the at least partially cured first layer, having a thickness and an area over the one of the one of the surface of the at least partially cured first layer which is greater than the minimum dimension; and

an electromagnetic beam directing device outputting a beam of electromagnetic energy capable of curing, into a solid form, at least a portion of the material of the droplet, the beam of electromagnetic energy having a width dimension equal to, or less than, the minimum dimension.

16. The apparatus of claim 15, wherein the at least one selectively openable nozzle includes at least a first nozzle in fluid communication with a first liquid, and a second nozzle in fluid communication with a second nozzle, and at least one component of the first liquid is different from any component of the second liquid.

17. The apparatus of claim 16, wherein the first and second liquids each contain living cells, and the living cells in the first liquid are different than the living cells in the second liquid.

18. The apparatus of claim 17, wherein the at least one selectively openable nozzle includes a third nozzle connected to a supply of liquid having a component thereof different than the components of the first liquid and the second liquid.

19. The apparatus of claim 16, further comprising a moveable stage configured to move the surface first under the liquid dispensing device and then under the electromagnetic beam directing device.

20. The apparatus of claim 19, wherein the moveable stage is moveable in a first direction extending in the direction of from the liquid dispensing device to the electromagnetic beam directing device, and a second direction crossing the first direction.

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