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Tunable 3D Droplet Self-Assembly for Ultra-High-Density Digital Micro-Reactor Arrays

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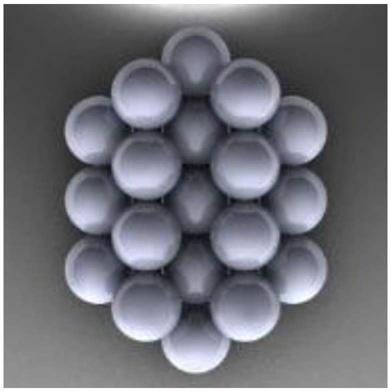
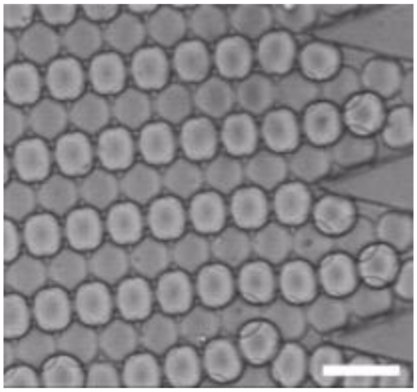
Electronic Supplementary Information #1



Video S 1 Colloidal Droplet Packing Illustration

Video Illustration of single droplet layer transitioning through H/D values of 1- 1.707 consisting of patterns ranging from (111) to (110) and finally (100).



Video S 2 Colloidal droplet packing (110) like formation

Video of colloidal droplet self-assembly in (110) like packing formation. Droplet diameter = 63 µm, chamber height = 82 µm, and w/o ratio is 65% generated at flow rates of 4.0 µL/min water and 2.15 µL/min heavy mineral oil. Scale bar is 100 µm.

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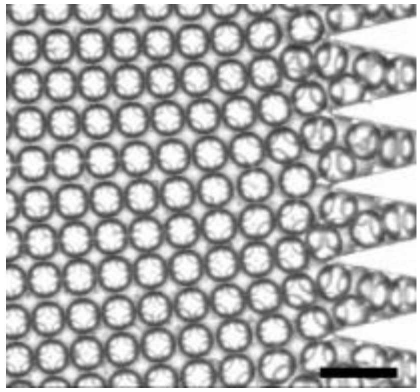
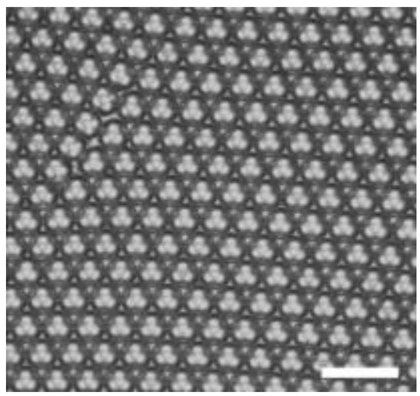


Video S 3 Colloidal droplet packing in (100) like formation

Video of colloidal droplet self-assembly in (111) like packing formation. Droplet diameter = 48 µm, chamber height = 78 µm, and w/o ratio is 63% generated at flow rates of 4.0 µL/min water and 2.35 µL/min heavy mineral oil. Scale bar is 100 µm



Video S 4 Colloidal droplet packing in (111) like formation

Video of colloidal droplet self-assembly in (111) like packing formation. Droplet diameter = 44 µm, chamber height = 80 µm, and w/o ratio is 66% generated at flow rates of 4.0 µL/min water and 2.0 µL/min heavy mineral oil. Scale bar is 100 µm.

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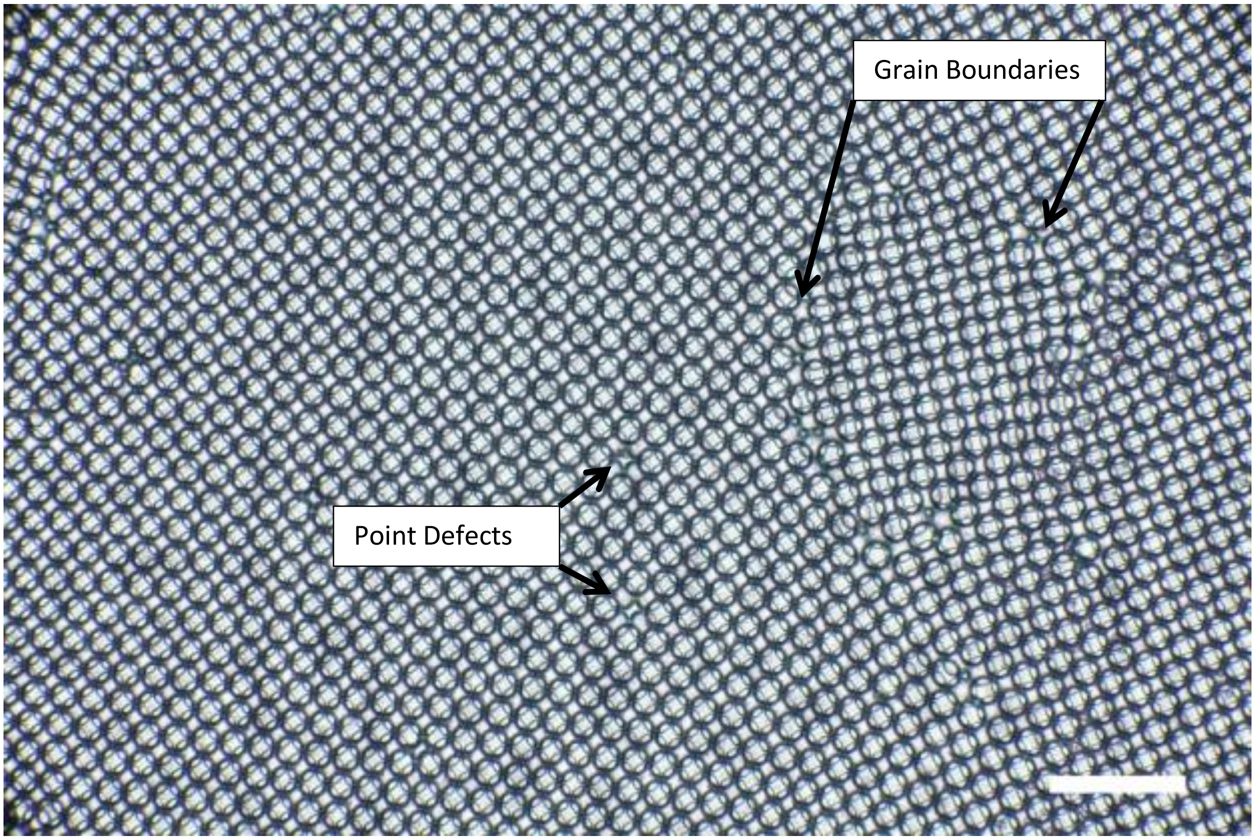

Fig. S 1 High-density double layer (100) brightfield image

Large field of view image of self-assembled droplets in (100) like droplet packing formation which demonstrates ability to form large uniform (100) lattice formations consisting of 44.5 µm droplets in 75 µm tall device, H/D = 1.68 and w/o ratio = 63%. Scale bar is 250 µm. Point defects and grain boundaries do occur when forming self-assembled colloidal lattice structures.

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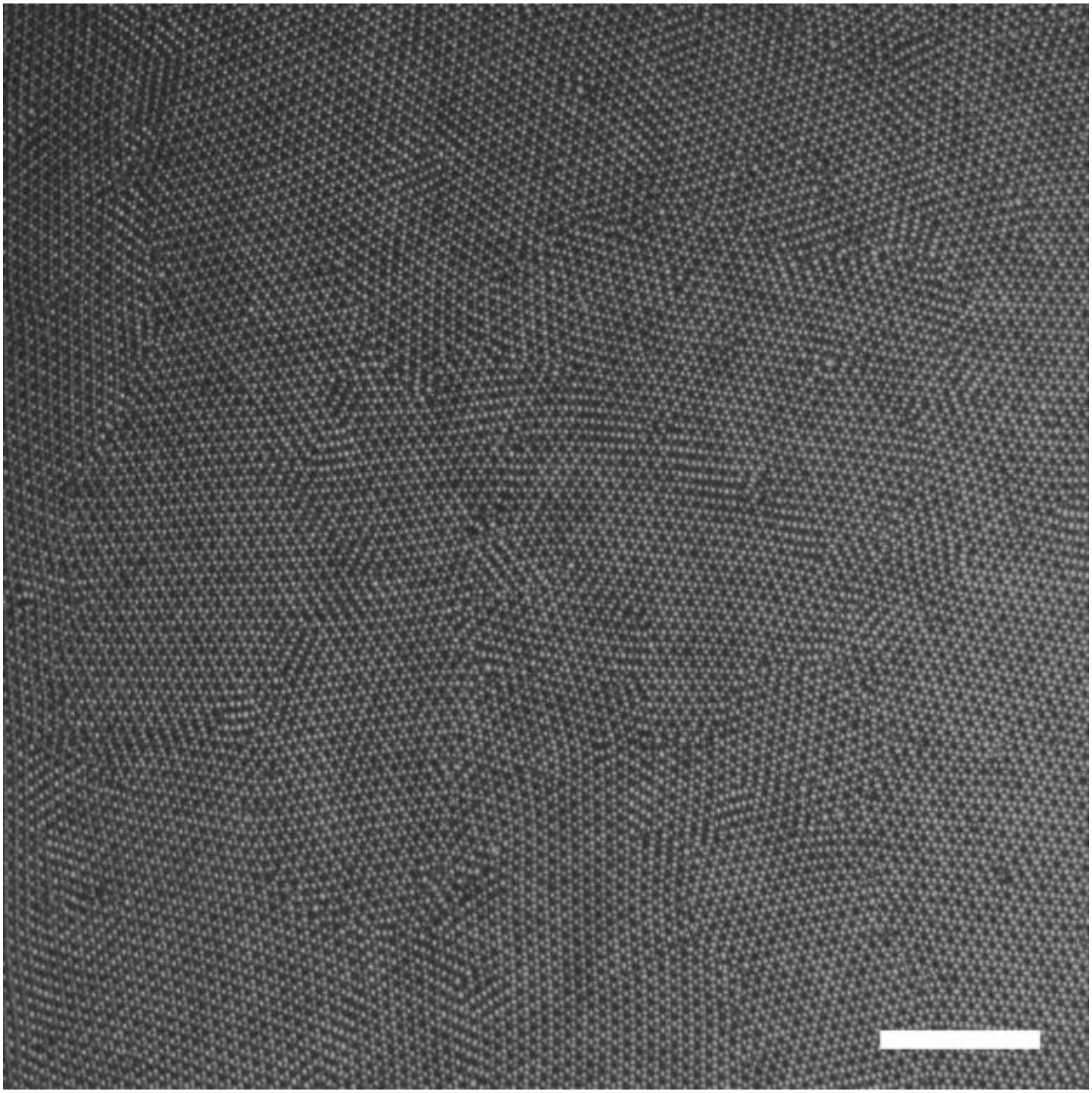

Fig. S 2 High-density double layer (111) brightfield image

Large field of view image of self-assembled droplets in (100) like droplet packing formation demonstrating ability to form large uniform (111) lattice formations. Droplet diameter = 42 µm, chamber height = 76 µm tall device, H/D = 1.81 and w/o ratio = 66%. Scale bar is 500 µm.

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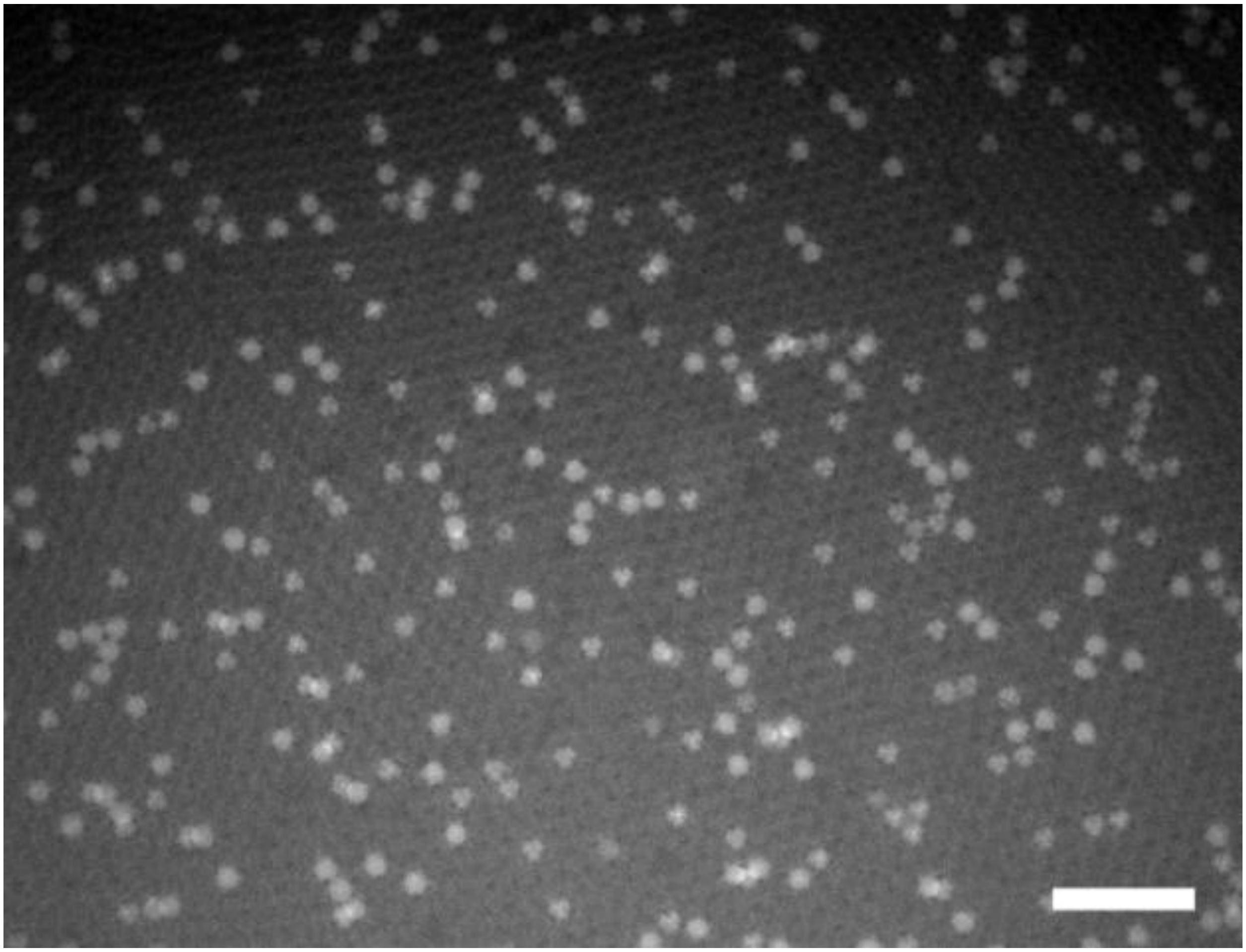

Fig. S 3 High-density double layer (111) fluorescence image

Large field of view Fluorescence image of 43 µm droplets in a 78 µm tall chamber packed in (111) like droplet packing formation with H/D of 1.81. Droplet coalescence occurs at a very small percentage and is undetectable in this image. Scale bar is 250 µm.

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Electronic Supplementary Information #2

Calculations of Crystalline Lattice Packing Parameters

Area of circle: $A_c = \pi r^2$

Volume of sphere: $V_s = \frac{4}{3}\pi r^3$

Unit Area of a rectangle: $A_r = l \times w$

Volume of a rectangular prism: $V_{rp} = l \times w \times w$

Unit Area of Hexagon enclosing spherical diameter D: $A_h = \frac{\sqrt{3}}{2}D^2 = 2\sqrt{3}r^2$

Unit hexagonal Volume enclosing spherical diameter D: $V_h = \frac{\sqrt{2}}{2}D^3 = 4\sqrt{2}r^3$

Volume of unit hexagonal prism enclosing spherical diameter D and height H: $V_{hp} = \frac{\sqrt{3}}{2}D^2 \times H$

Droplet Packing and Effective Row Spacing

As monodisperse-sized droplets close-pack into confined geometries, they adapt characteristics common of colloidal crystalline packing and form rows of droplets with minimal spacing between them. Close-packed droplet formations self-assemble into repeatable patterns as a result of their spherical geometries and interactions with the bounding surfaces. By controlling the height of a large chamber area relative to the droplet diameter, you can predictively tune the amount of overlap allowed between adjacent rows of droplets. The effective row spacing, D_x, between the rows of droplets in multilayer planes can be calculated using Pythagorean's theorem and is demonstrated in Fig. S1. Here the main parameter is droplet diameter D, and the droplet radius, r.

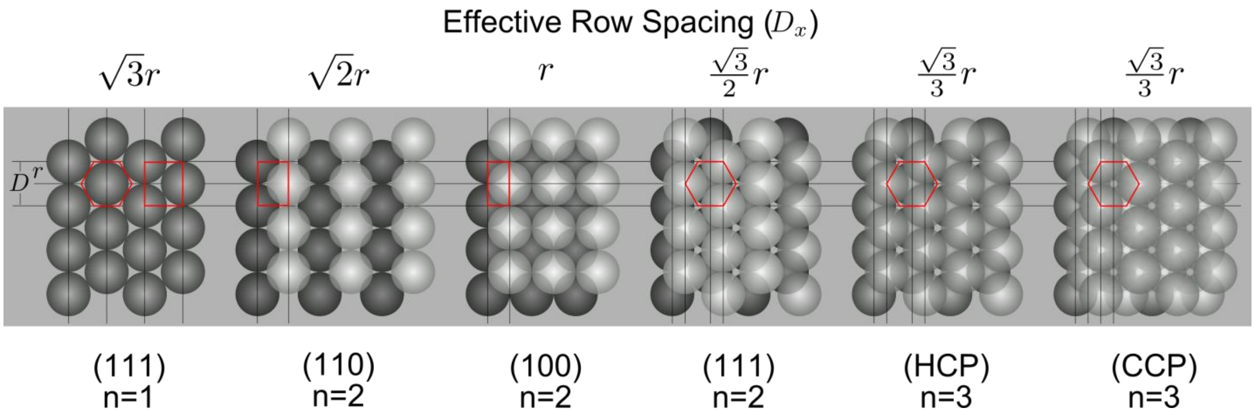


Fig. S1: *Row spacing of droplet packing configurations. The red outlines indicate the unit area on the sensor that would contain a single droplet which is a function of the droplet diameter and effective row spacingD_x. The single layer, n=1, (111) formation can have a unit droplet area described using either a hexagonal shape as shown on the left side, or a rectangular shape as shown on the right. For n>1 (111) formations it is more appropriate to describe the unit area as a hexagonal shape, thus for all (111) formations, a hexagonal shape will be used and for all (110) and (100 formations a rectangular shape will be used.*

Based on Pythagorean's theorem the minimal spacing between two adjacent droplets is one droplet diameter, D, related to the displacement in the x, y, and z directions denoted here as the scalar valuesD_x,D_y, and D_z in the following relationship.

$$D_x{}^2 + D_y{}^2 + D_z{}^2 = D^2 \qquad \textit{eq. (1)}$$

Here we assign scalar values in the x, y, and z directions based on crystalline packing structure as follows:

D = the droplet diameter and defines the minimal spacing between any two adjacent droplets

D_x = the droplets lateral displacement between rows in the x direction

$D_y = D/2$, close packed lattice planes in (111), (110), and (100) configurations have adjacent rows displaced by exactly one droplet radius in the y direction orthogonal to the row displacementD_x.

$z = H - D$, The droplets' vertical displacement orthogonal to D_x and D_y which is confined by controlling the chamber height, H, relative to the droplet diameter D.

Substitution into eq. (1) and solving for D_x yields:

$$D_x = \sqrt{D^2 - \left(\frac{D}{2}\right)^2 - (H-D)^2} \qquad \textit{eq. (2)}$$

For n=1 (111) configurations, let H=D

Substitution into eq. (2) and simplification yields:

$$D_x = \sqrt{D^2 - \left(\frac{D}{2}\right)^2 - 0^2} = \frac{\sqrt{3}}{2}D$$

For n layers the average row spacing changes inversely to n as D_x (111,n) = $\frac{\sqrt{3}}{2n}D$ for all n. Note this does not necessarily indicate the exact spacing of each row, but rather it is used to indicate on average how far in the x direction would you extend a rectangle with length D in the y direction to detect 1 droplet volume in an n layer packing configuration.

For n=2 (110) configurations let H=D/2

Substitution into eq. (2) and simplification yields:

$$D_x = \sqrt{D^2 - \left(\frac{D}{2}\right)^2 - \left(\frac{D}{2}\right)^2} = \frac{\sqrt{2}}{2}D$$

For n layers the average row spacing changes inversely to n as D_x (110,n) = $\frac{\sqrt{2}}{n}D$

For n=2 (100) configurations let H=$\sqrt{2}/2D$

Substitution into eq. (2) and simplification yields:

$$D_x = \sqrt{D^2 - \left(\frac{D}{2}\right)^2 - \left(\frac{\sqrt{2}D}{2}\right)^2} = \frac{1}{2}D$$

For n layers the average row spacing changes inversely to n as D_x (100,n) = $\frac{D}{n}$

Sensor Area Coverage and Area Overlap

Sensor area-coverage efficiency and sensor area overlap are used to describe what percentage of a unit area on the sensor is covered by a single droplet, multiple overlapping droplets, or no droplet at all. Fig. S2 illustrates the subtle nuances in these parameters and summarizes the main values derived for each. For simplicity, it is more intuitive to describe a unit area on the sensor in all (111) hexagonal like packing formations using a hexagonal shape, and for (110) or (100) packing formations to use a rectangular packing shape. For n=1 (111) formations, a rectangular shape can be used to describe a single droplet's unit area coverage, as seen in Fig. S1, but it is less convenient when working with n>1 formations.

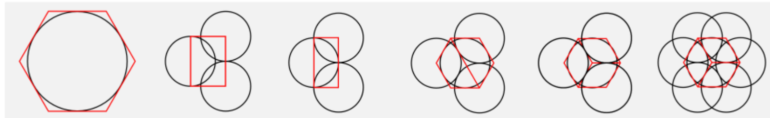
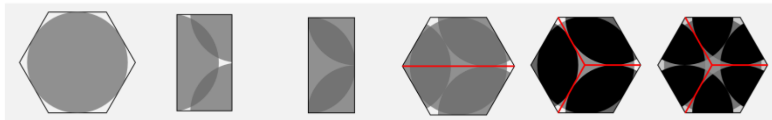
Orientation	(111) n=1	(110) n=2	(100) n=2	(111) n=2	(HCP) n=3	(CCP) n=3
Unit Sensor Area Shape						
Unit Sensor Area (D·Dx)	$\frac{\sqrt{3}D^2}{2}$	$\frac{\sqrt{2}D^2}{2}$	$\frac{D^2}{2}$	$\frac{\sqrt{3}D^2}{4}$	$\frac{\sqrt{3}D^2}{6}$	$\frac{\sqrt{3}D^2}{6}$
Sensor Overlap Regions light grey = 1 dark grey = 2 black = 3						
Sensor Area Coverage	$\frac{\pi D^2}{4}$	0.6948 D^2	0.500 D^2	0.4224 D^2	0.2816 D^2	0.2887 D^2
Area Coverage Efficiency (%)	90.07	98.26	100	97.56	97.56	100
Total Sensor Overlap Area (%)	0	12.81	57.08	83.82	92.69	90.69
Double Sensor Overlap Area (%) (Dark Grey Area)	0	12.81	57.08	83.82	13.31	6.87
Triple Sensor Overlap Area (%) (Black Area)	0	0	0	0	83.82	79.38

Fig. S2: *Sensor Area Coverage efficiency and Area overlap are described for each unit area that is occupied by a single droplet (outlined in red). For n>1 (111) hexagonal packing formations the unit area is drawn the same but is subdivided into n subsections as indicated by the red lines.*

Sensor area coverage and overlap area is determined by drawing the droplets in their respective configurations and integrating the area of each respective parameter, then dividing by the total area to determine the percentage values. For the simplest case of n=1 (111) packing configuration, the calculation is performed mathematically as follows:

For n=1 (111) area coverage ratio:

Ratio of unit circle area, A_c, to unit Hexagon area, A_h, is

$$\frac{A_c = \pi r^2}{A_h = 2\sqrt{3}r^2} \rightarrow \pi\frac{\sqrt{3}}{6} = 0.9069$$

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Droplet Area and Volume Overlap

Droplet Area overlap and volume overlap is defined from the perspective of the imaging plane and only considers the area occupied by the droplet itself without regard to empty space around it. Fig. S3 illustrates each packing configuration and the relevant parameters.

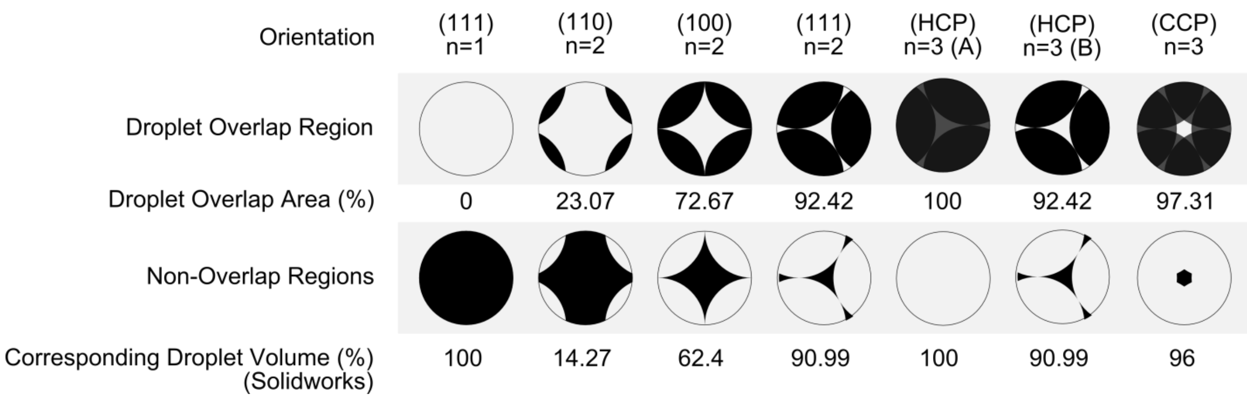

Fig. S3: *Droplet area overlap and volume overlap are determined based on the region of a droplets area that is overlapping with adjacent droplets.*

Droplet area overlap area is determined by drawing the droplets in their respective configurations and integrating the area of the overlapping regions then dividing by the total droplet area to determine the percentage values. The volume of the overlapping droplet areas in the different configurations were determined using Solidworks to perform an embossed cut through a droplet sphere to remove sections of overlapping volume and measuring the remaining volume of the droplet segments using the mass analysis tool. Fig. S4 illustrates some simple images of the spherical volumes

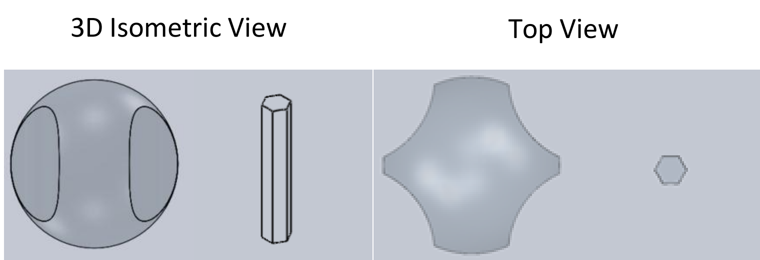

Fig. S4: *Droplet volume measurements were calculated by integrating the volumetric properties of 3D spherical shapes corresponding to non-overlapping droplet areas. These volumes were then divided by the original spherical volume to determine what percentage of the droplet volume is overlapping with other droplets.*

Water/oil (w/o) Volume Ratio

Unit hexagonal volume

Water/oil volume ratio, w/o, for a droplet emulsion is defined the volume of a spherical droplet, V_s, relative to its surrounding media in which it is contained. For close packed configurations of monodisperse spherical colloidal lattices or droplet emulsions in a cubic close packing, CCP, configurations, the smallest unit volume that contains a droplet defines the greatest w/o volume ratio achievable. In the case of the droplet emulsion, when a droplet is completely surrounded by adjacent droplets, 12 in all, a unit hexagonal cell can be drawn to enclose the droplet with radius r. In the case where the droplets are influenced by surrounding bounding boxes which limit the packing ability of droplets, lower w/o volume ratios are obtained. For the case of a sphere in a unit hexagonal volume the w/o volume ratio is described as follows:

$$volume\ ratio\ (w/o) = \frac{V_s = \frac{4}{3}\pi r^3}{V_h = 4\sqrt{2}r^3} \qquad eq.\ (3)$$

Solving for eq. (3) yields a maximum w/o volume ratio for n=∞ spherical packing of:

$$w/o\ = \frac{V_s = \frac{4}{3}\pi r^3}{V_h = 4\sqrt{2}r^3} \rightarrow \pi\frac{\sqrt{2}}{6} = 0.7405$$

This is the theoretical maximum for n=∞ droplet layers in a spherical droplet packing configuration.

In the case of a flat top and bottom surface, the volume characterization is modified to use a hexagonal prism as the unit volume as shown in Fig. S5.

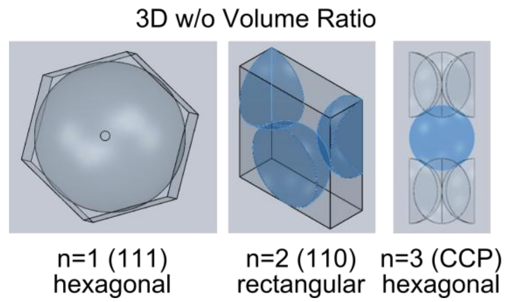

Fig. S5: W/O *Droplet volume measurements were calculated by integrating the volumetric properties of 3D droplet configurations relative to the volumes they occupy in the microreactor chamber.*

Single (111) volume ratio for n=1

Volume Ratio of a sphere, V_s, to a unit hexagonal prism volume, V_{hp}, is defined as:

$$w/o = \frac{V_s = \frac{4}{3}\pi r^3}{V_{hp} = \frac{\sqrt{3}}{2}D^2 \times H} \qquad eq.\ (4)$$

Substitution H=D into eq. (4) and solving yields:

$$w/o = \frac{V_s = \frac{4}{3}\pi r^3}{V_{hp} = \frac{\sqrt{3}}{2}D^2 \times D} \rightarrow \pi\frac{\sqrt{3}}{9} = 0.6046$$

Thus the volume ratio is 60.46% for n=1 (111) single layer lattice packing

Double (111) where n=2

For n lattice planes, there will be a total of n layers with n droplets occupying the same hexagonal area thus eq. (4) can be modified to divide the hexagonal area by n, or multiply the number of droplets by n as follows:

$$w/o = \frac{V_s = \frac{4}{3}\pi r^3 \times n}{V_{hp} = \frac{\sqrt{3}}{2}D^2 \times H} \qquad eq.\ (5)$$

Substitution of H=(1+$\sqrt{2/3}$)D and n=2 into eq. (5) yields:

$$w/o = \frac{V_s = \frac{4}{3}\pi r^3 \times 2}{V_{hp} = \frac{\sqrt{3}}{2}D^2 \times (1 + \sqrt{2/3})D} \rightarrow \pi\frac{2(\sqrt{3} - \sqrt{2})}{3} = 0.6657$$

Thus the volume ratio is 66.57% for n=2 (111) double layer lattice packing

Triple (111) where n=3

Substitution of H=(1+2$\sqrt{2/3}$)D and n=3 into eq. (5) yields

$$w/o = \frac{V_s = \frac{4}{3}\pi r^3 \times 3}{V_{hp} = \frac{\sqrt{3}}{2}D^2 \times (1 + 2\sqrt{2/3})D} \rightarrow \pi\frac{2\sqrt{2} - \sqrt{3}}{5} = 0.6889$$

Thus the volume ratio is 68.89 % for a (111) triple layer lattice

For n layers of (111) packed droplets

There will be n droplets per unit hexagonal prism volume and the hexagonal prism height for n layers is defined as H=(1+$(n-1)\sqrt{2/3}$)D) based on Pythagorean's theorem, substitution into eq. (5) and simplification yields:

$$w/o = \frac{V_s = \frac{4}{3}\pi r^3 \times n}{V_{hp} = \frac{\sqrt{3}}{2} D^2 \times (1 + (n-1)\sqrt{2/3})D} \rightarrow \pi \frac{n\sqrt{2}}{6n + 3\sqrt{6} - 6} \qquad eq.\ (6)$$

Alternative n layer (111) calculations using rectangular prism geometry

As mentioned above, either a hexagonal shape or a rectangular shape may be used to calculate the unit area for a droplet in (111) packing configurations. Using this approach, one would define the volume of a rectangular prism, V_{rp}, as dependent on droplet diameter D, effective row spacing D_x, and chamber height H. Thus the volume ratio of a droplet sphere in a unit rectangular prism is described as follows:

$$w/o\ volume\ ratio = \frac{V_s = \frac{4}{3}\pi r^3}{V_{rp} = D \times D_x \times H} \qquad eq.\ (7)$$

Substituting the same H(111,n)=(1+$(n-1)\sqrt{2/3}$D) and D_x (111,n) = $\frac{\sqrt{3}}{2n}D$ from above into eq. (7) and simplifying yields:

$$\frac{w}{o} = \frac{V_s = \frac{4}{3}\pi r^3}{V_{rp} = D \times \frac{\sqrt{3}}{2n} D \times (1 + (n-1)\sqrt{\frac{2}{3}}\mathrm{D})} \rightarrow \pi \frac{n\sqrt{2}}{6n + 3\sqrt{6} - 6} = eq. (6)$$

This value is exactly the same as eq. (6) above indicating that the use of either hexagonal or rectangular unit-area shape descriptors are viable solutions for determining, Sensor area coverage, w/o volume ratio, and droplet density and is further demonstrated in the density calculations.

For n=∞ layers of (111)

Substitution of n=∞ into eq. (6) and solving yields:

$$As\ n \rightarrow \infty, \quad \frac{w}{o} = \pi \frac{n\sqrt{2}}{6n + 3\sqrt{6} - 6} \rightarrow \pi \frac{\sqrt{2}}{6} = 74.05$$

which is identical to the previous solution of eq. (3) that describes the theoretical maximum w/o volume ratio for a sphere bound in a unit hexagonal volume.

Double (110) where n=2

As mentioned above, the unit area coverage for a sphere in (100) or (111) packing configurations is best described using a rectangular shape which bisects 1 half sphere of one row and two quarter spheres in the adjacent row. For n=2 droplet layers, a unit rectangular prism with side lengths of droplet diameter D, row spacing D_x, and chamber height H will contain 1 droplet volume. Thus the w/o volume ratio of a sphere, V_s, in a unit rectangular prism with volume, V_{rp}, is defined as:

$$w/o\ volume\ ratio = \frac{V_s = \frac{4}{3}\pi r^3}{V_{rp} = D \times D_x \times H} \qquad eq.\ (7)$$

Substituting H=1.5D and D_x= $\sqrt{2}r$ into eq. (7) and solving yields:

$$w/o = \frac{V_s = \frac{4}{3}\pi r^3}{V_{rp} = D \times \sqrt{2}r \times 1.5D} \rightarrow \pi \frac{\sqrt{2}}{9} = 0.4937$$

Thus the volume ratio is 49.37% for an n=2 (110) double layer droplet lattice reactor array.

For n layers of (110)

Similar to the pattern for (111) packing formation, one can expect that the number of droplets per unit rectangular prism volumes will increase proportional to n. Thus the unit area can be divided proportional to n, or the number of droplets per can be multiplied by a factor of n. In the case of (110), and (100) lattice formations, it doesn't make sense to describe the condition of n=1 as it will not predictably self-assemble into that formation. However to since there are n≥2 layers, it is still useful to define the equation in terms of n for those cases. For n layers in (110) packing configurations, we substitute H=(1+$(n-1)$/2)D and D_x(110,n)= $\frac{\sqrt{2}}{n}D$, as calculated above, into eq. (7) then simplify to yield:

$$\frac{w}{o} = \frac{V_s = \frac{4}{3}\pi r^3}{V_{rp} = D \times \frac{\sqrt{2}}{n} D \times \left(1 + \frac{n-1}{2}\right) D} \rightarrow \pi \frac{n\sqrt{2}}{6(n+1)} for\ n \geq 2 \qquad eq.\ (8)$$

For n=∞ layers of (110)

Substitution of n=∞ into eq. (8) and solving yields:

$$As\ n \rightarrow \infty, \qquad \pi \frac{n\sqrt{2}}{6(n+1)} \rightarrow \pi \frac{\sqrt{2}}{6} = .7405$$

which is identical to the previous solution of eq. (3) that describes the theoretical maximum w/o volume ratio for a sphere bound in a unit hexagonal volume.

Double (100) where n=2

For (100) packing configurations the case is very similar to (110) packing formations therefore we substitute H=(1+$\sqrt{2}$/2)D and D_x= r into eq. (7) to yield:

$$\frac{V_s = \frac{4}{3}\pi r^3}{V_r = D \times r \times (1 + \frac{\sqrt{2}}{2})D} \rightarrow \pi \frac{2-\sqrt{2}}{3} = 0.6134$$

Thus the volume ratio for n=2 (100) droplet layers is 61.34%

For n layers of (100)

Similar to the (110) lattice formation condition, we solve the general case of n≥2 (100) layers by substituting D_x (100,n) = $\frac{D}{n}$, as calculated above, and H=(1+$(n-1)\sqrt{2}/2$)D, based on Pythagorean's theorem, into eq. (7) and simplify to yield:

$$w/o = \frac{V_s = \frac{4}{3}\pi r^3 \times n}{V_{rp} = D \times D \times \left(1 + (n-1)\frac{\sqrt{2}}{2}\right) D} \rightarrow \pi \frac{n\sqrt{2}}{6\left((n-1)+\sqrt{2}\right)} for\, n \geq 2 \qquad eq. (9)$$

For n=∞ layers of (100)

Substitution of n=∞ into eq. (9) and solving yields:

$$As\, n \rightarrow \infty, \qquad \pi \frac{n\sqrt{2}}{6((n-1)+\sqrt{2})} \rightarrow \pi \frac{\sqrt{2}}{6} = 0.7405$$

which is identical to the previous solution of eq. (3) that describes the theoretical maximum w/o volume ratio for a sphere bound in a unit hexagonal volume.

Droplet Packing Density

Packing density is defined as the inverse of the unit droplet area, A_u, based on droplet diameter, D, and effective row spacing, D_x, as follows:

$$Density = \frac{1\, drop}{A_u} = \frac{1\, drop}{D \times D_x} \qquad eq. (10)$$

For n multiples, the density increases proportional to n since there would be n increases in droplet layers within the same area. For this reason, one can reasonably determine the density of n droplet layers by multiplying the density of an n=1 layer by n.

Using previous calculations for D_x for each packing configuration and substituting into eq. (1)0 yields the following results:

For n=1 single (111) droplet layers we substitute D_x = $\sqrt{3}r$ into eq. (1)0 and let the droplet diameter D = 46 x 10^{-3} mm's to yield a density value of:

$$Density = \frac{1}{A_u} = \frac{1}{(46 \times 10^{-3}\, mm)^2 \times \frac{\sqrt{3}}{2}} = 546\, drops \cdot mm^{-2}$$

For n (111) droplet layers the effective row spacing D_x(111,n) becomes $\frac{\sqrt{3}}{n}r$, as calculated above, which substituted into Eq. (10) yields:

$$Density = \frac{1}{A_u} = \frac{n}{(46 \times 10^{-3}\ mm)^2 \times \frac{\sqrt{3}}{2}} = n \times 546\ drops \cdot mm^{-2} \qquad eq.\ (11)$$

Thus substitution of n=2 and 3 into eq. (11) yields the following results:

Density for n=2 yields 1091 $drops \cdot mm^{-2}$

Density for n=3 yields 1637 $drops \cdot mm^{-2}$

For n=2 (110) droplet layers we substitute $D_x = \sqrt{2}r$ into eq. (10) and let the droplet diameter D = 46 x 10^{-3} mm's to yield a density value of:

$$Density = \frac{1}{A_u} = \frac{1}{(46 \times 10^{-3}\ mm)^2 \times \frac{\sqrt{2}}{2}} = 668\ drops \cdot mm^{-2}$$

For n=2 (100) droplet layers we substitute $D_x = r$ into eq. (10) and let the droplet diameter D = 46 x 10^{-3} mm's to yield a density value of:

$$Density = \frac{1}{A_u} = \frac{1}{(46 \times 10^{-3}\ mm)^2 \times \frac{1}{2}} = 945\ drops \cdot mm^{-2}$$

Electronic Supplementary Material (ESI) for Lab on a Chip
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Tunable 3D Droplet Self-Assembly for Ultra-High-Density Digital Micro-Reactor Arrays

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Electronic Supplementary Information #3

Fluorescence Imaging Analysis and Radial Intensity Profile Calculations

Captured Fluorescence images such as those shown in Fig. S1 were processed and analyzed using ImageJ software[1] to perform detection, characterization, digital quantification, and radial profile analysis of droplets present.

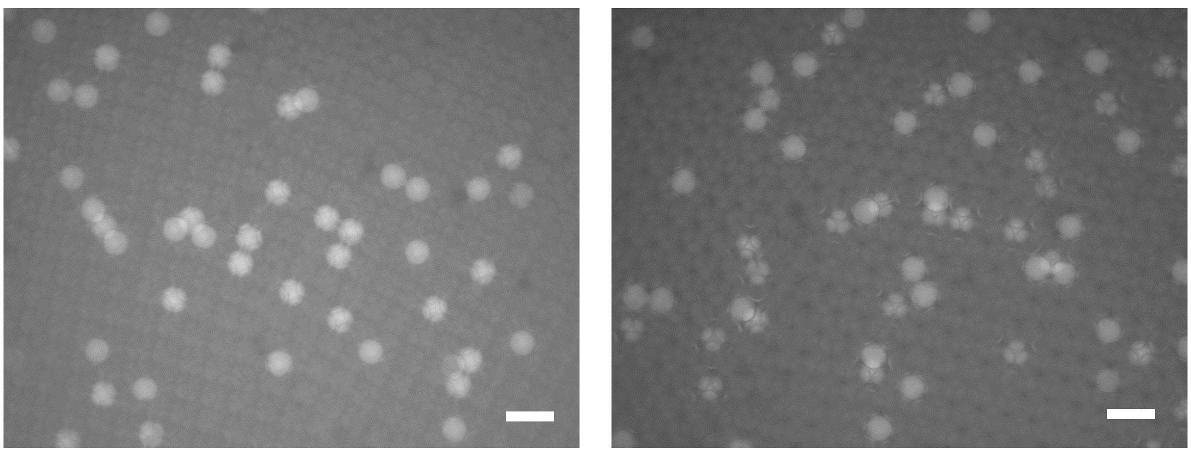

Fig. S1: *Fluorescence images of n=2 double layer (100) left, and (111) right, packing configurations. Scale bars =100 µm.*

First the subtract background, tool was used with a rolling ball radius of 50 pixels followed by a brightness and contrast adjustment setting the scale min and max pixel values from 0 to 100 respectively to yield the images shown in Fig. S2.

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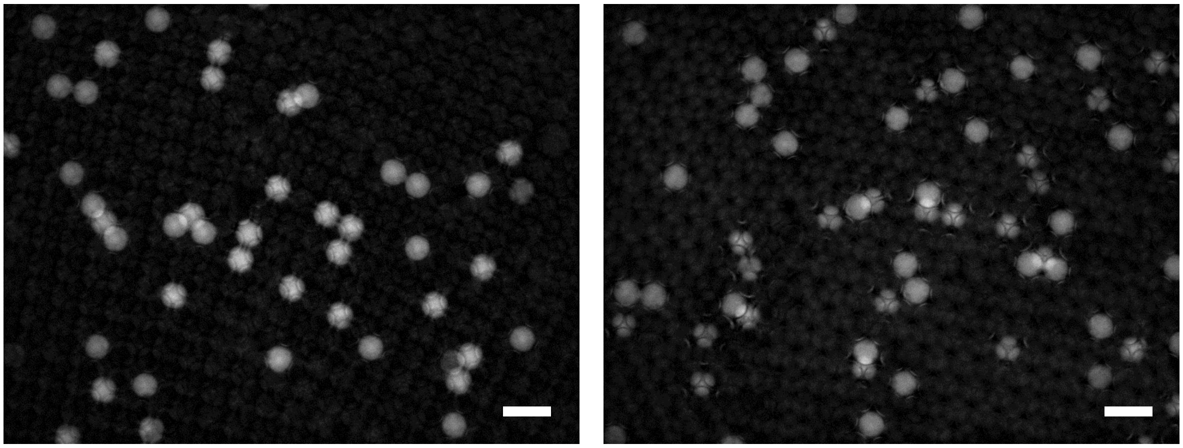

Fig. S2: *Fluorescence images found in Fig. S1 after background subtraction and contrast enhancement. Scale bars =100 µm.*

The greyscale images were converted to binary by thresholding then, using a built in binary watershed isolation tool, overlapping droplets were isolated as shown in Fig. S3. Using the built in ImageJ particle analyzer tool the size, location, and intensity of the fluorescent droplets were determined and automatically counted to quantify the positive droplets relative to total droplets present in the imaging plane as shown in Fig. S4.

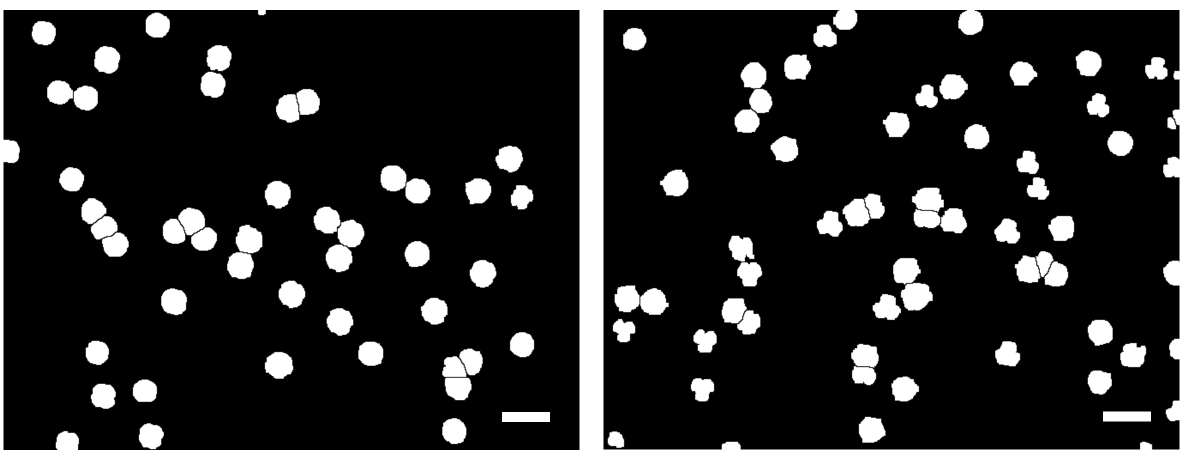

Fig. S3: *Binary images after thresholding and watershed separation of overlapping droplets illustrating droplet detection and localization of n=2 double layer (100) left, and (111) right, packing configurations. Scale bars =100 µm.*

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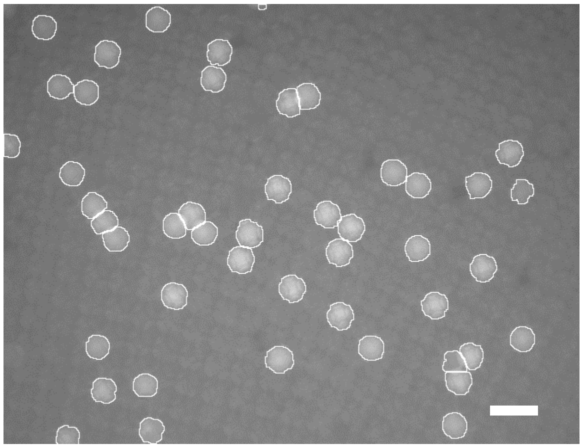
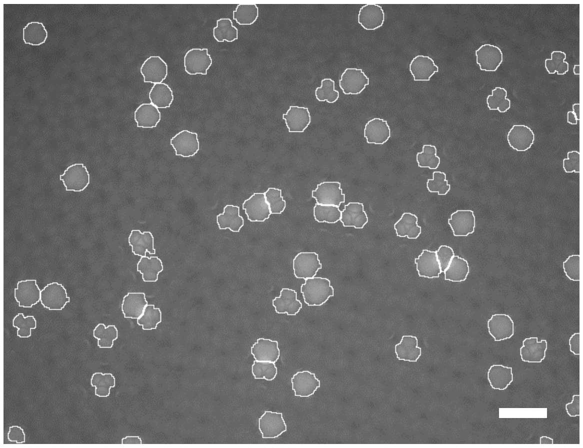
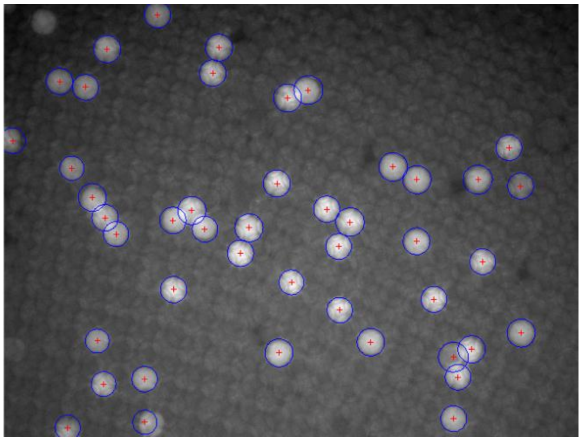

Fig. S4: Original i*mages with label overlays illustrating droplet detection and localization of n=2 double layer (100) bottom and top left, and (111) top right, packing configurations. The analyze particle tool was used to characterize droplet intensity and percentage of amplified droplets.* A similar processing techniques were performed using custom Matlab code to develop the resulting image seen in the bottom row. *Scale bars =100 µm.*

Radial Profile Plots

Radial intensity profiles are determined using Image J software for droplets within each layer of the various packing configurations as illustrated in Fig. S5. The centroid locations and average size information captured from the ImageJ particle analyzer tool were used to quickly select droplets and analyze their radial profile plots using an ImageJ plugin[2]. The output includes two columns of data which are then plotted as radial distance in pixels on the horizontal axis and integrated normalized intensity on the vertical axis.

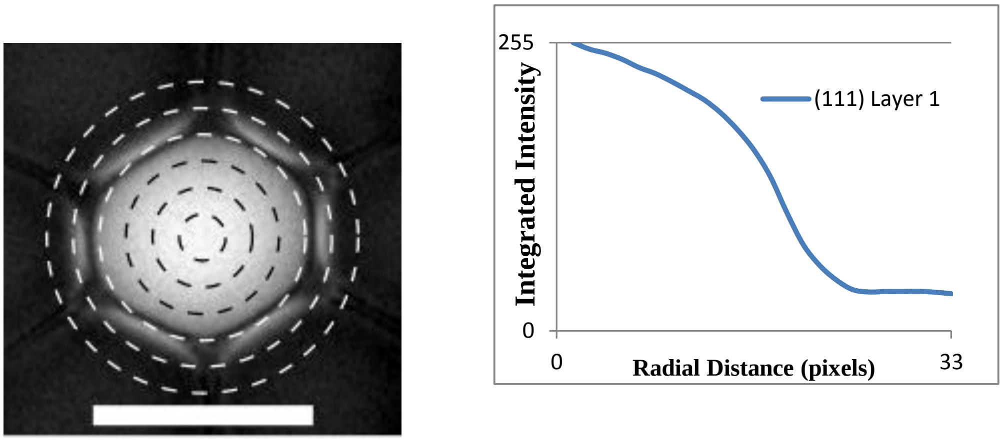

Fig. S5: *Radial profile of fluorescent droplets. Left: image of droplet in n=1 (111) lattice structure with concentric rings drawn in 25% intervals from centerline, scale bar is 50 µm's or 22 pixels. Right: Profile intensity integrated radially outward from centerline to 150% beyond outer droplet diameter, 33 pixels, in 1 pixel increments to yield the radial profile plot of the droplet on the left.*

Each radial profile plot was non-dimensionalized to percentage values on the horizontal axis by dividing by the droplet radius. Similarly the fluorescence intensity on the vertical axis was corrected to a relative intensity by dividing by the mean maximum intensity of positive droplets in the first layer of each packing configuration. Fig. S6 shows individual radial profile plots of droplets in the first layer from all five different lattice formations. For n>1 configurations, the fluorescence intensity of droplets in second or third layer planes were adjusted relative to the top layer to preserve comparison of image intensities across the differing packing configurations. Measurements of several droplets in their respective lattice positions of top, middle, or bottom planes in n=1,2, or 3 layers were averaged together for each packing configuration and compiled into a single plot for comparison as shown in Fig. S5 in the main manuscript(N≥2). This was done to determine how detrimental droplet overlap is in n>1 packing configurations and be able to compare overall relative intensities from underlying droplet planes.

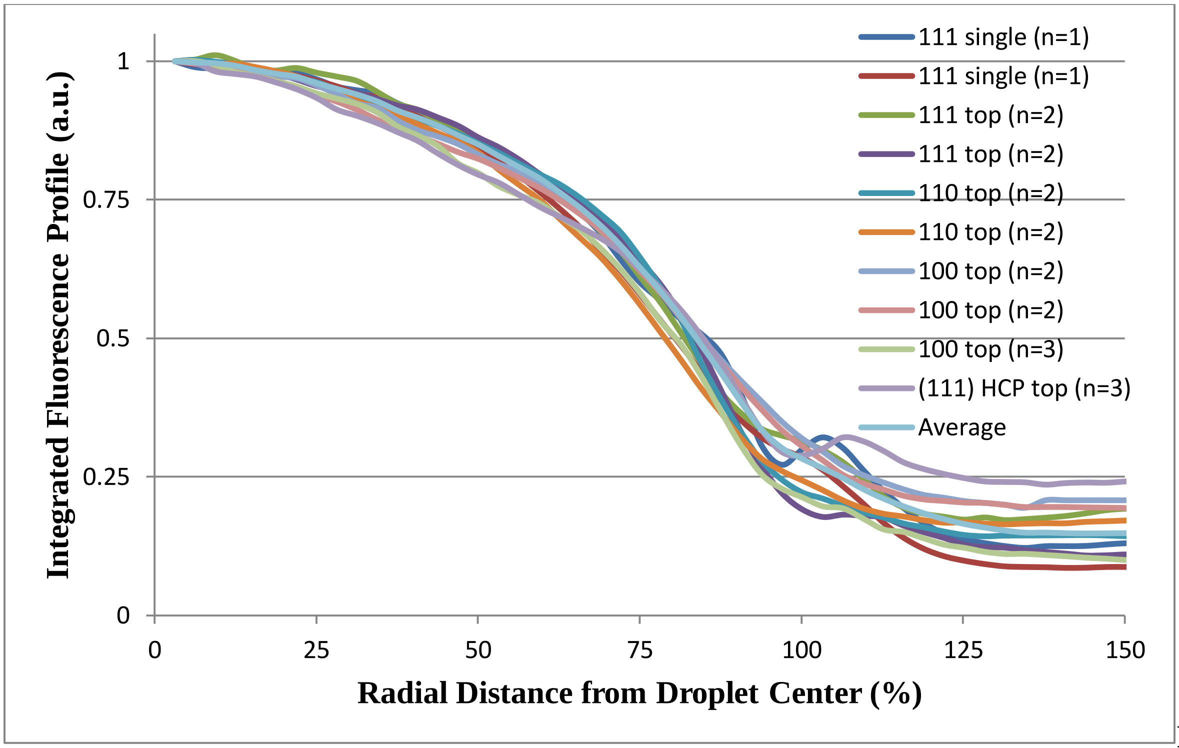

Fig. S6: *Individual and averaged radial profile plots of top layer droplets from each packing configuration for comparison of uniformity of fluorescence intensity across all packing conformations.*

The variation in background intensities seen on the right end in Fig. S6, beyond 100% radial distances, are likely the result of variations in imaging exposures, poor flatfield imaging performance, variations in PCR amplification across runs, and increased changes in background scatter resulting from different packing configurations.