Beating Poisson encapsulation statistics using close-packed ordering†

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Loading drops with discrete objects, such as particles and cells, is often necessary when performing chemical and biological assays in microfluidic devices. However, random loading techniques are inefficient, yielding a majority of empty and unusable drops. We use deformable particles that are close packed to insert a controllable number of particles into every drop. This provides a simple, flexible means of efficiently encapsulating a controllable number of particles per drop.

Drops formed in microfluidic devices are useful for chemical and biological assays.[1–6] The drops can serve as picoliter vessels within which individual reactions can be performed. With microfluidic devices, the drops can be formed, merged, and sorted at kilohertz rates.[7] This combination of speed, containment, and small volumes is very useful for many applications, such as screening libraries of unknown chemical compounds, evolving cells and enzymes, and analyzing genetic material.[8–14] All such applications require the encapsulation of cells, beads, and other discrete reagents in the drops. However, current methods to encapsulate objects into drops are very inefficient.[15] Typically, this is accomplished by diluting suspensions of the materials and encapsulating into the drops at random; the resulting Poisson statistics lead to a large number of empty drops with a much smaller number having a single particle. This can be wasteful, negating the speed and efficiency afforded by droplet microfluidics.

This inefficiency has stimulated the development of new methods that provide more efficient encapsulation.[16] For example, a laser can be used to guide particles, ensuring single particle encapsulation.[17] This method affords superb control for the encapsulation of objects over a range of sizes – from beads tens of microns in diameter to cells and cellular organelles only a few microns in diameter. However, this method is very difficult to use, requiring sophisticated optical equipment and extensive participation by the user. Moreover, it is slow, having a maximum speed of only a few hertz. Alternatively, inertial ordering can be used to passively organize particles prior to encapsulation.[15] Under appropriate flow conditions inertial effects lead to regular spacing of the particles; by matching the periodicity of the drop formation with the periodicity of the particles, efficient encapsulation can be achieved. While simple and fast, this method is not robust and is very difficult to implement. An optimal system for loading a controlled number of particles into drops would combine the robust operation and control of laser guidance with the speed and automation of inertial ordering; however, such a method has not been reported.

In this paper, we introduce a simple, robust method to load a controllable number of particles into every drop. When the volume fraction of the particles is increased to the point that they are close-packed, they naturally order into a regular spacing, providing a periodic flow of particles; by matching the periodicity of the drop formation to the particle flow, we achieve near perfect loading of a prescribed number of particles in each drop. By using slightly deformable particles, we avoid clogging, providing a robust, simple method for controlling particle loading in drops.

We use compliant gel particles in these experiments.[18] The compliance of the particles prevents clogging of the channels. The gels are useful substrates for chemical and biological applications.[19–22] For example, the gel particles can be functionalized with a variety of compounds, including fluorophores, DNA fragments, antibodies, and enzymes. This makes them useful for many functions: as optical elements for labeling; as substrates for reactions; and as collection sites for products secreted by cells. Because they are deformable they can be packed at near 100% volume fraction without clogging. It is this high volume fraction that enables the particles to order into a regular spacing, which is essential to achieve uniform filling of the drops.

The gel particles themselves are synthesized using microfluidic droplet polymerization.[18] They consist of 10% polyacrylamide with 10% cross-linker. They have an average diameter of 30 μm with a polydispersity of 5%. To encapsulate the gel particles, we use a microfluidic device consisting of two cross-channel junctions arranged in series, Fig. 1a. In the first junction we add water to space the particles, while in the second junction we add oil to encapsulate the particles, as shown in Fig. 1b. All our microfluidic devices are fabricated using standard soft-lithographic techniques.[23]

To demonstrate the periodic particle flow that results from their close packing, we monitor the particles at the drop-formation junction. We inject the 30 μm particles into a drop maker with channels that are 25 μm tall. Because the particle diameters exceed the height of the channel, they are confined in a monolayer. Because the particles are close packed, they naturally order into a hexagonal array, as shown in Fig. 2a. As the particles approach the drop-formation junction, the channel narrows to the width of one particle; this causes particles to flow single-file as they enter the drop formation junction, as shown in Fig. 2a. To measure the periodicity of the particle flow we record movies at 50,000 frames-per-second using a Phantom V7 camera. We measure the average grayscale intensity in the drop formation junction in the region demarcated by the box in Fig. 2a. Because the particles are brighter than the background we observe a spike in the intensity as a function of time as a particle moves through the box, as shown in Fig. 2b. The spikes are periodic, reflecting the periodicity of the particles, as shown in Fig. 2b; this results from their close-packed ordering.

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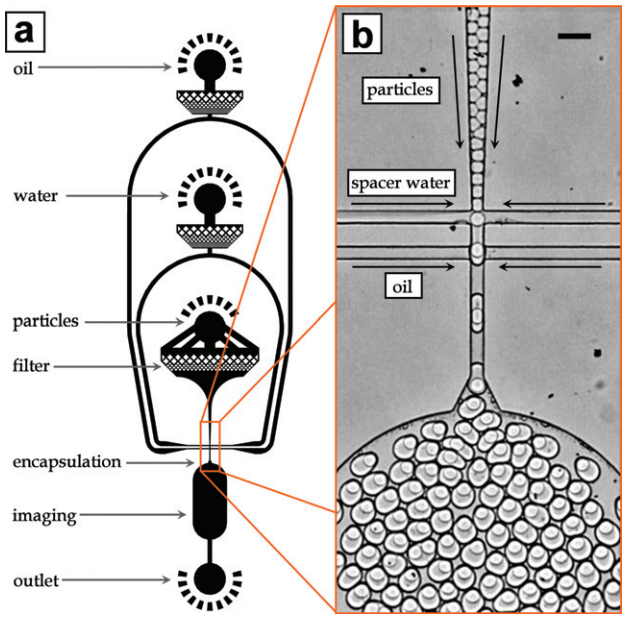

Fig. 1 (a) Schematic of encapsulation device. (b) Photomicrograph of particle encapsulation. The particles are injected at high volume fraction, causing them to order. Water is added in the first junction to space the particles prior to encapsulation. Oil is added in the second junction to form drops and encapsulate the particles. The scale bar denotes 50 μm.

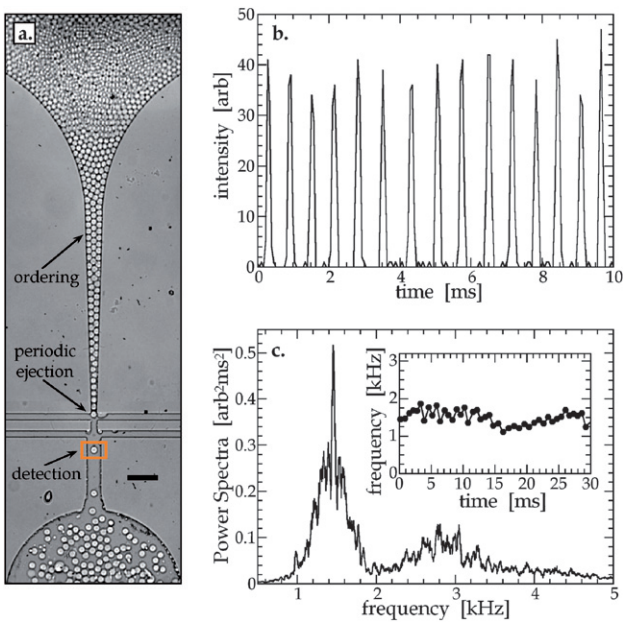

Fig. 2 (a) Photomicrograph of close-packed gel particles flowing into encapsulation junction. The evenly spaced particles flow at a periodic rate into the encapsulation junction. The scale bar denotes 100 μm. (b) Average grayscale intensity in the box demarcated; each spike corresponds to a particle moving through the box. (c) Power spectrum of the intensity time trace, with a sharp peak at the average frequency of 1.5 kHz. The frequency as a function of time is plotted inset.

We determine the frequency of the particles by measuring the period between consecutive peaks, and plot the result in the inset of Fig. 2c. The frequency is very nearly constant with an average of 1.5 kHz; the drift in the frequency is due to slight polydispersity of the particles and variation of flow rates due to finite accuracy of the pumps. To demonstrate that the periodic flow is maintained over the long term, we compute the power spectrum of the intensity time trace, Fig. 2c; the power spectrum is a time average over the full operation of the device and is thus representative of its long-term periodicity. The power spectrum has a sharp spike at 1.5 kHz, demonstrating this frequency dominates over the long term, as shown in Fig. 2c. This constant periodicity ensures that the particles can be efficiently encapsulated into the drops; moreover, this efficiency persists over long times.

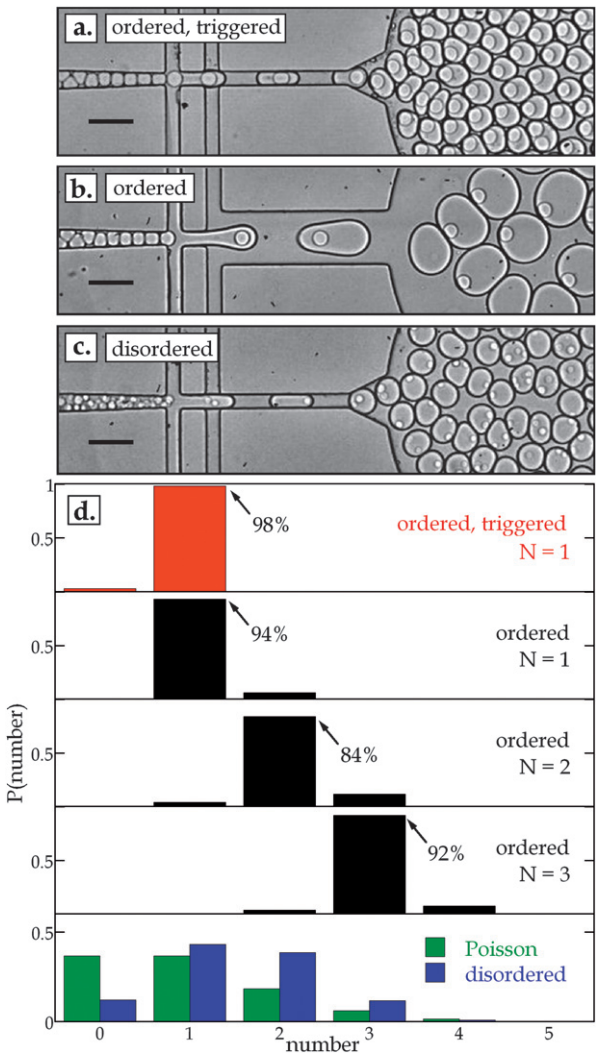

Fig. 3 Encapsulation of close-packed particles. Photomicrograph of encapsulation utilizing close-packed ordering and droplet triggering (a), close-packed ordering (b), and in which particles are disordered and encapsulated inefficiently (c). The scale bars denote 75 μm. (d) Probability distributions of the number of particles per drop. We obtain the highest encapsulation efficiency using both ordering and triggering, though ordering alone provides reasonable encapsulation efficiency, as shown for one, two, and three particles. Standard methods using random encapsulation (Poisson) are far less efficient, as is the case in which the particles are disordered.

To encapsulate the ordered particles we adjust the flow of oil in the drop-making junction to form drops at the same rate as that of the

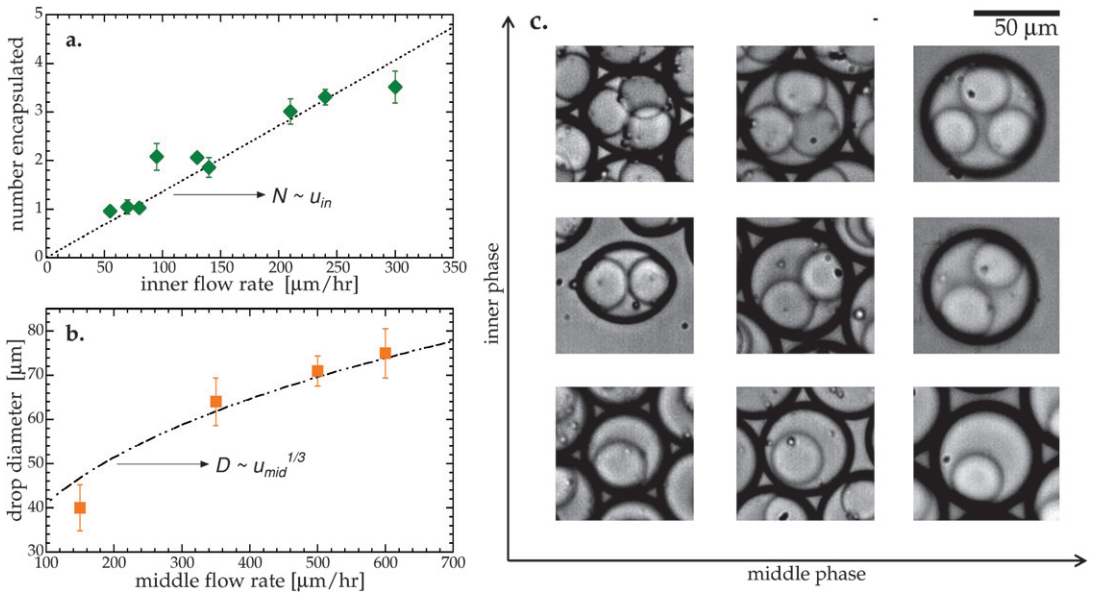

Fig. 4 Control of particle number and drop size. (a) Number of particles encapsulated as a function of inner phase flow rate. (b) Drop diameter as a function of middle phase flow rate. (c) Photomicrographs of different encapsulation morphologies obtained by adjusting the inner and middle phase flow rates. The continuous phase flow rate is held constant at 600 μl/h.

particles. To match the periodicities, we adjust either the flow rate of the inner phase, which adjusts the particle periodicity, or the flow rate of the continuous phase, which adjusts the drop formation periodicity. However, even with flow rates optimized, the periodicities can drift over time due to compliance in the device and finite accuracy of the pumps; this can lead to improper loading of some drops. To overcome this limitation and enable even higher encapsulation efficiency we also implement a triggered drop formation. We use a long thin nozzle to form drops and encapsulate the particles; this nozzle is of similar width to that of the particle diameter, as shown in Fig. 3a and in the movie available online.† Thus, the particles plug the nozzle when they are in position for encapsulation; this triggers drop formation, thereby allowing it to compensate for drift in the periodicity of the particles, leading to near perfect encapsulation efficiency, as shown by the measurement of the number distribution in Fig. 3d.

The utility of triggered drop formation is limited to the insertion of a single particle in each drop. To increase the number of particles encapsulated per drop we use a wider nozzle, precluding the triggering of drop formation by the particles, as shown in Fig. 3b and in the movie available online.† Instead, we rely solely on close-packed ordering of the particles to encapsulate a fixed number in each drop. The encapsulation efficiency remains very good; far better than with random ordering of the particles, as shown by comparison of the ordered and disordered cases in Figs. 3b–c. This is highlighted directly by the measured number distribution, which has an amplitude of 0.94, as shown in Fig. 3d. This device allows us to encapsulate multiple particles per drop. By adjusting the drop period to an integer multiple of that of the particles, we encapsulate two particles per drop with 0.84 probability, while three particles per drop are encapsulated with 0.92 probability.

This method of encapsulating particles into drops has several attractive features; because the particles are close-packed, density fluctuations are minimized and efficient encapsulation can be maintained over the long term. Because particle periodicity can be controlled independently of drop formation, a controlled number of particles can be inserted into every drop. Moreover, the suspension can be diluted as desired *in situ* to select the final volume fraction of the drop. Such control is extremely important when using encapsulation in combination with other processes, such as drop merger and sorting, which can be very sensitive to drop size and periodicity. To demonstrate this control over encapsulation we adjust the number of particles encapsulated and the volume fraction of the drop. To control particle number, we adjust the inner phase flow rate; the number of particles encapsulated per drop is linear with this flow rate, as shown in Fig. 4a. To control drop size and volume fraction, we adjust the middle phase flow rate; the diameter of the drop scales with this flow rate, as shown in Fig. 4b. Thus, we are able to independently control both the number of particles encapsulated and the volume of the drops, as exemplified by the images in Fig. 4c. This is possible with a range of particle sizes, from a few microns to several hundred microns in diameter.

This method is a simple, flexible, and robust technique for loading particles into drops. It allows the number of particles encapsulated, the size and volume fraction of the drops in which they are encapsulated, and the frequency and flow velocity of the drops to each be controlled independently. This flexibility should ease integration with other processes, such as drop merger and sorting, which are very sensitive to flow conditions and drop properties. It can also be used to encapsulate cells efficiently into drops by first encapsulating the cells into gels and then encapsulating the gels into drops. Thus, this method should prove very useful in implementing efficient encapsulation into lab-on-a-chip devices.

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