**Definitions:**

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| Emulsion | a fine dispersion of minute droplets of one liquid in another in which it is not soluble or miscible. |
| Microfluidics | Microfluidics refers to the behavior, precise control, and manipulation of fluids that are geometrically constrained to a small scale at which surface forces dominate volumetric forces. It is a multidisciplinary field that involves engineering, physics, chemistry, biochemistry, nanotechnology, and biotechnology. [Wikipedia](https://en.wikipedia.org/wiki/Microfluidics) |
| Surfactant | Surfactants are compounds that lower the surface tension between two liquids, between a gas and a liquid, or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, or dispersants. The word "surfactant" is a blend of surface-active agent, coined c. 1950. [Wikipedia](https://en.wikipedia.org/wiki/Surfactant) |
| Poisson Statistics | In [probability theory](https://en.wikipedia.org/wiki/Probability_theory) and [statistics](https://en.wikipedia.org/wiki/Statistics), the **Poisson distribution** ([/ˈpwɑːsɒn/](https://en.wikipedia.org/wiki/Help:IPA/English); French pronunciation: ​[[pwasɔ̃]](https://en.wikipedia.org/wiki/Help:IPA/French)), named after [French](https://en.wikipedia.org/wiki/France) mathematician [Denis Poisson](https://en.wikipedia.org/wiki/Sim%C3%A9on_Denis_Poisson), is a [discrete probability distribution](https://en.wikipedia.org/wiki/Discrete_probability_distribution) that expresses the probability of a given number of events occurring in a fixed interval of time or space if these events occur with a known constant mean rate and [independently](https://en.wikipedia.org/wiki/Statistical_independence) of the time since the last event. |
| monodisperse emulsion | Emulsion where all droplets are equal in volume/mass/spacing? |
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**Points:**

* 1. “ddPCR technology uses a combination of microfluidics and proprietary surfactant chemistries to divide PCR samples into water-in-oil droplets (Hindson et al. 2011).”
  2. “In droplet dPCR, uniform partition is a crucial prerequisite for high accuracy due to the premise of the Poisson distribution of target DNA fragments. Besides, the number of partitions directly influences the resolution, accuracy and dynamic range of a dPCR assay.”

Droplet generation is no joke.

1. “Bio-Rad's Droplet Digital PCR System automates the ddPCR workflow of droplet generation, thermal cycling, droplet reading, and data analysis, making this technology accessible to the working research laboratory. “ - Sounds like they already have one in market. Why are we designing one?
2. “high-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions, allowing for the sensitive detection of rare targets and enabling a ±10% precision in quantification” - What does this mean?
3. “Load the plate into the QX200 droplet reader and start your run. Each well is read serially. Droplets are sipped and the singulator unpacks the emulsified droplets and streams them in single file past a two-color optical detection system to determine which droplets contain a target and which do not”. - This seems complex.
4. “The aqueous solution ejected out of the MiCA will be continuously pinched off at the nozzles and then completely transformed into small droplets that fly into the receiving oil to form w/o emulsion. By tuning the spinning speed and changing the MiCA with different channel numbers or sizes, we are able to generate droplets of various sizes.” - Have they confirmed that their generated droplets are uniform in shape/size/mass?
5. “The formulation of the two immiscible phases is critical to the characteristics and stability of w/o emulsion. First, the density of the oil has to be lower than that of water, thus the received flying droplets will sink into the bottom of the tube during centrifugation. However, the density difference between oil and water needs to be small, providing enough buoyancy to ensure that the droplets do not merge together. Second, low viscosity (ca. 10 cSt) of the oil is preferred, ensuring that the aqueous droplets can easily enter the oil–air interface without being smashed. Third, the formula of the oil and surfactant should be PCR-compatible”
6. “This method, however, is still facing a bottleneck – the detection scheme of serial detection is still far from being ideal or elegant. Furthermore, exposure of the amplified DNA product to the experimental environment is very likely to interfere in quantitative DNA assays afterwards and such contamination needs to be completely eliminated for medical research and diagnosis.” - I see.

**Equipment Wishlist:**

1. Imaging system for looking at droplet generation. We need to be able to see that the droplets are forming correctly.
2. Droplet generation machine + droplet imaging machine for teardown analysis.
3. PCR machine for doing PCRs.
4. Gold standard for comparison/verification?

**Other Considerations:**

1. In addition to uniform droplet generation, target molecule distribution within each droplet is important.
2. The CLEARdPCR software is garbage ([their code](https://github.com/MengchengJ/CLEAR_code)) never ask me to do anything with it please.