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New High-Performance Droplet Freezing Assay (HP-DFA) for the Analysis of Ice Nuclei with Complex Composition

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Freezing of water above homogeneous freezing is catalyzed by ice nucleation active (INA) particles called ice nuclei (IN), which can be of various inorganic or biological origin. The freezing temperatures reach up to -1 °C for some biological samples and are dependent on the chemical composition of the IN. The standard method to analyze IN in solution is the droplet freezing assay (DFA) established by Gabor Vali in 1970. Several modifications and improvements were already made within the last decades, but they are still limited by either small droplet numbers, large droplet volumes or inadequate separation of the single droplets resulting in mutual interferences and therefore improper measurements.

The probability that miscellaneous IN are concentrated together in one droplet increases with the volume of the droplet, which can be described by the Poisson distribution. At a given concentration, the partition of a droplet into several smaller droplets leads to finely dispersed IN resulting in better statistics and therefore in a better resolution of the nucleation spectrum.

We designed a new customized high-performance droplet freezing assay (HP-DFA), which represents an upgrade of the previously existing DFAs in terms of temperature range and statistics. The necessity of observing freezing events at temperatures lower than homogeneous freezing due to freezing point depression, requires high-performance thermostats combined with an optimal insulation. Furthermore, we developed a cooling setup, which allows both huge and tiny temperature changes within a very short period of time. Besides that, the new DFA provides the analysis of more than 750 droplets per run with a small droplet volume of 5 μL. This enables a fast and more precise analysis of biological samples with complex IN composition as well as better statistics for every sample at the same time.