

## **TINA, a new fully automated high-performance droplet freezing assay coupled to a customized infrared detection system**

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Heterogeneous ice nucleation is frequently investigated by simultaneously cooling a defined number of droplets of equal volume in droplet freezing assays. In 1971, Gabor Vali established the quantitative assessment of ice nuclei active at specific temperatures for many droplet freezing assays. Since then, several instruments have been developed, and various modifications and improvements have been made. However, for quantitative analysis of ice nuclei, the current known droplet freezing assays are still limited by either small droplet numbers, large droplet volumes, inadequate separation of the single droplets, which can result in mutual interferences, or imprecise temperature control within the system.

Here, we present the Twin Ice Nucleation Assay (TINA), which represents an improvement of the until now existing droplet freezing assays in terms of temperature range and statistics. Above all, we developed a distinct detection system for freezing events in droplet freezing assays, where the temperature gradient of each single droplet is tracked individually by infrared cameras coupled to a self-written software. In the fully automated setup, ice nucleation can be studied in two independently cooled, customized aluminum blocks run by a high-performance thermostat. We developed a cooling setup, which allows both huge and tiny temperature changes within a very short period of time, combined with an optimal insulation. Hence, measurements can be performed at temperatures down to  $-55\text{ }^{\circ}\text{C}$  (218 K) and at cooling rates up to  $3\text{ K min}^{-1}$ . Besides that, TINA provides the analysis of nearly 1000 droplets per run with various droplet volumes between  $1\text{ }\mu\text{L}$  and  $50\text{ }\mu\text{L}$ . 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.