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## Microwave assisted freezing

### Part 2: Impact of microwave energy and duty cycle on ice crystal size distribution

**Mathieu SADOT, Sébastien CURET, Sylvie CHEVALLIER, Alain LE-BAIL, Olivier ROUAUD, Michel HAVET**

#### Abstract

Among innovative freezing processes, electromagnetic wave assistance during freezing has received great attention in recent years. The literature provides interesting studies indicating that smaller ice crystals may be obtained during freezing with the assistance of microwaves. In the companion paper, we designed a laboratory scale prototype to perform experiments of this type in well controlled conditions at 2.45 GHz. In the current study, we performed freezing experiments using this prototype and developed a protocol to measure ice crystal sizes using X-ray micro-tomography. The methodology was first validated by measuring ice crystal distribution in unidirectional conventional freezing. Then several microwave assisted freezing modalities were studied. Experiments were conducted with pulsed and continuously applied microwaves. The impact of microwave irradiation time was studied at a constant incident microwave power, and then at constant energy supplied to the system. We observed a significant reduction of up to 25% in ice crystal size when microwave assistance was used during freezing with both pulsed and continuously applied microwaves. These observations tend to disconfirm the hypothesis in the literature according to which ice crystal size reduction is due to temperature oscillations. The only remaining hypothesis in the literature is that ice crystal size reduction may be due to the perturbation of the H-bond network in water, which could be a precursor to crystalline structure. It appeared that at constant incident microwave power, the increase in irradiation time tended to decrease ice crystal size. No significant difference in ice crystal size reduction was observed for a constant energy supplied to the system. The amount of energy supplied by microwaves seems to be a factor influencing ice crystal size.

#### Keywords

Freezing; microwaves; ice crystals; micro-tomography

## Highlights

Ice crystal size is measured by X-ray micro-tomography after freeze-drying.  
The X-ray micro-tomography method is validated for conventional freezing.  
Microwave assistance during freezing significantly reduces ice crystal size (-25%).  
Ice crystal size reduction is not due to temperature oscillations.  
Increasing the amount of energy supplied by microwaves decreases ice crystal size.

## Industrial relevance

Microwave assisted freezing is an emerging process which permits reducing ice crystal size, and thus improving frozen product quality. The measurement of ice crystals in samples frozen in controlled conditions of both freezing and microwave irradiation allows studying the main parameters that induce the reduction of their size. Better understanding of the relevant factors influencing nucleation and/or crystal growth can help to optimize the process.

## 1. Introduction

Freezing is a well-known process used in industry to preserve food product quality by reducing reaction rates (Lim et al., 2004) and controlling microbial activity (Bremer & Ridley, 2004). Usually, fast freezing processes are used because they induce small ice crystals, giving rise to better quality (Devine et al., 1996). Indeed, larger crystals can damage cell membranes because of the pressure exerted by water expansion during phase change, which results in cracks and mass losses during thawing (Bevilacqua et al., 1979; Delgado & Sun, 2001). As fast freezing processes are energy-demanding because they require very low temperatures, such as in cryogenic processes (Dempsey & Bansal, 2012), it is challenging to reduce ice crystal size without increasing energy consumption, using innovative processes. The assistance of mechanical agitation, pressure shift, ultrasounds, magnetic fields and AC/DC electric fields during freezing were studied to control ice nucleation (Dalvi-Isfahan et al., 2017; Jha et al., 2017; Le Bail et al., 2002; Woo & Mujumdar, 2010). Electromagnetic wave assistance during freezing was also investigated. Smaller ice crystals and better ultrastructural preservation were found in squid retina frozen at 83 K with microwave assistance (2.45GHz) (Hanyu et al., 1992). The authors' hypothesis to explain ice crystal size reduction was that the rotation of water molecules caused by the alternative electric field induced by microwaves interferes in the H-bond network that could be a precursor of the crystalline structure. Later, Jackson et al.(1997) observed less ice and more glass in an aqueous ethylene glycol solution frozen under 2.45 GHz microwave irradiation. The assistance of a lower frequency during freezing

was studied on a real food product. Smaller crystals and better quality were obtained for pork frozen with a liquid nitrogen spray under radiofrequency assistance (27.12 MHz) (Anese et al., 2012). A reduction of 75% in ice crystal size was found in trout frozen by cold air convection under radiofrequency assistance (27.12 MHz) in comparison with conventional freezing (Hafezparast-moadab et al., 2018). Smaller ice crystals were also observed on a real food product during microwave (2.45 GHz) assisted freezing of pork meat (Xanthakis et al., 2014). These authors proposed that temperature oscillations due to microwave pulses could induce the partial melting of crystals and therefore secondary nucleation, leading to more crystals of smaller dimensions. The European FREEZEWAVE project (*FP7-ERA-Net SUSFOOD*) was launched due to this diversity of results and the lack of information on the physical mechanisms involved in the improvement of the freezing process by microwaves. The goals of this project were to assess the efficiency of microwave assistance in improving the frozen product quality by reducing the ice crystal and help understanding the underlying physical phenomena.

As the microwave assisted freezing process is rather complex, numerical simulations were considered as a promising alternative to obtain better understanding of the physical phenomena involved (Sadot et al., 2017). The companion paper confirmed the major benefits of modelling. It provided information on the thermal mechanisms and the interactions between microwaves and matter occurring during the phase change. The simulation results were used to design a laboratory scale prototype and define the correct product dimensions in order to observe the impact of microwave assistance on frozen product quality. Consequently, the aim of the current study is to investigate the relationship between microwave assistance during freezing and ice crystal size, in very well defined experimental conditions applied to a food model. One of the main challenges consists in implementing a rigorous methodology to measure ice crystal size.

## 2. Material and methods

### 2.1. Product

The companion paper dedicated to the numerical modelling pointed out the major influence of the food product's thermophysical and dielectric properties on the physical phenomena involved, especially the thermal kinetics. To ensure that accurate and reproducible results were obtained, the experiments were conducted on a homogeneous product whose composition was perfectly known and almost constant for each experiment. For this reason, we chose a methylcellulose gel prepared using a well-defined protocol, described in detail in the companion paper. This gel is made of 13% methylcellulose powder (Tylose H 100000 YP2, ShinEtsu, Jp) and 87% water. This solution was poured

into a polyether ether ketone (PEEK) mould and stored at 4°C for at least 10 h before the freezing experiments.

## 2.2. Experimental device

To study the impact of microwave assistance on ice crystal size during freezing, the experiments were performed on the experimental device presented in the companion paper. Freezing was ensured by gaseous nitrogen under controlled temperature before its entry in the applicator. The latter was located in a rectangular waveguide WR 340 (section 86 mm x 43 mm), to ensure the propagation of the fundamental single-mode TE<sub>10</sub> at a frequency of 2450 MHz. The samples used in this study had the same width (9 mm) and length (39 mm) as those used in the companion paper. However, a shorter height (3 mm) was chosen to minimize the heterogeneity of microwave treatment within the product due to the exponential attenuation of microwaves with penetration depth. Furthermore, a 1 mm high polystyrene layer was placed above the sample to decrease the difference in thermal treatment between the surface and the bulk of the product (Figure 1).

## 2.3. Experimental protocol

The methylcellulose gel had to be taken out of the cold chamber at least 1 h before the experiment to ensure a homogeneous temperature at experimental room ambience. It was then placed in the applicator with its mould and the polystyrene isolation. Once the inlet temperature of gaseous nitrogen was stable ( $-47^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ), it was introduced in the waveguide and the microwave generator was turned on. At the end of the experiments, the applicator flange was removed from the waveguide and directly introduced in a cold chamber at  $-18^{\circ}\text{C}$  where the sample was extracted before being freeze-dried. The experiments performed to determine the impact of microwaves on ice crystal size were repeated at least 5 times per modality.

## 2.4. Ice crystal size measurement by X-ray micro-tomography

### 2.4.1. Freeze-drying

Freeze-drying was used to sublimate ice crystals in vapour to keep crystal ghosts without structure modification. The samples were placed directly in the Flexi-Dry freeze-dryer (FTS Systems, USA) after extraction. The samples were freeze-dried under vacuum at 40 mTorr (5.33 Pa) at  $-85^{\circ}\text{C}$  for at least 20 h. We did not observe any mass fluctuation greater than 0.1% of the initial mass after 18 h on larger samples. We noted that the sample volume did not change after freeze-drying, indicating no shrinkage.

### 2.4.2. Sample scan

After freeze-drying the sample was cut in its centre to obtain a 5 mm square parallelepiped (Figure 2).

The cut sample was then placed in the Skyscan1174v2 (Bruker microCT, Kontich, Belgium) tomograph and scanned. X-ray power was set to 50 kV and 800  $\mu$ A. The CCD (charged coupled device) detector has a resolution of 1024 x 1024 pixels. The exposure time was set to 1600 ms and the pixel size to 6.46  $\mu$ m, the lowest value allowed by this apparatus. The acquisition was the mean of 2 pictures taken every 0.1°, over 180°.

#### 2.4.3. Reconstruction

Sample reconstruction was done using 2D projections with a NRecon 1.7.1.0 (Bruker microCT, Kontich, Belgium) using the Feldkamp algorithm (Feldkamp, 1984). The regions of interest (ROI) treated of at least 500 × 500 pixels were selected from the entire sample height. The distance between each slice was 1 pixel (6.45  $\mu$ m). To convert the image into grey levels from 0 to 255, an intensity interval proportional to X-Ray attenuation ranging from 0.00015 to 0.035 was selected. A ring artefact reduction of 20 (maximum) was used to avoid noise on the reconstructed images.

#### 2.4.4. Image treatment

Once the 3D reconstruction had been done, the images needed a further treatment step with a CTAn 1.16.4.1 (Bruker microCT, Kontich, Belgium) before analysis. First, the grey level images were thresholded to create 2 pixel categories: the clearer ones corresponding to the solid matrix, the darker ones corresponding to the air (cavities of ice crystal shape). Thresholding was done automatically by the Otsu method. The resulting binary images, containing only black and white pixels, were processed further to convert the few aberrant white pixels not linked to the main structure (Figure 3).

#### 2.4.5. Image analysis

The treated binary images were analysed with the CT-analyser software (Bruker microCT, Kontich, Belgium) to estimate the cavities resulting from ice sublimation, representative of ice crystal sizes. Local cavity size was measured according to the definition given by Hildebrand and Ruegsegger (Hildebrand & Ruegsegger, 1997), initially created to determine the solid matrix thickness. First a skeletisation step is performed. The skeleton is defined by all the black pixels equidistant from the solid matrix (white pixels). Then, for all the skeleton pixels, the cavity size is defined as the diameter of the largest sphere fulfilling two conditions: the pixel is in the sphere (not necessarily the centre), and the sphere only includes black pixels. This principle is illustrated in Figure 4. Skeleton pixel no. 1 gives a sphere of equivalent diameter corresponding to sphere A, pixels 2 and 3 to sphere B, pixel 4 to sphere C and pixel 5 to sphere D. This illustration is a 2D representation of the principle which in fact works in 3 dimensions. The advantage of this method is that it reduces the noise due to the orientation of the 3D structure to the minimum (Ulrich et al., 1999).

### 3. Results and discussion

#### 3.1. Validation of the methodology in a 1D freezing experiment without microwave

Ice crystal size was measured by X-ray micro-tomography after freeze-drying of the frozen samples. To estimate the relevance of this methodology, we first applied it in a condition, without microwaves, for which the thermal kinetic was known to have an impact on ice crystal size. We performed experiments on a thicker sample (25 mm) with unidirectional freezing from top to bottom of the sample. As shown in Figure 5, the global ice crystal size distribution is Gaussian; it follows a normal law as in most food freezing (Bevilacqua & Zaritzky, 1982). The mean of the normal distribution is thereafter defined as the mean equivalent diameter. The mean equivalent diameter was of the expected order of magnitude. The ice crystal size distribution was close to what was found in the literature, in gelatine by image analysis of histological slices (Woinet, Andrieu, Laurent, et al., 1998), and in microproteins by X-ray micro-tomography (Mousavi et al., 2005). After this initial indication of the relevance of the method, we focused on the evolution of crystal size as a function of sample depth. The total volume of interest (VOI) was then subdivided into 8 sub-volumes of 3 mm height to measure the local ice crystal size (Figure 6). Results at VOI 1 and VOI 8 should be considered with circumspection due to difficulties in the cutting procedure leading to edge effects. Despite this latter point, the measurements clearly show smaller ice crystals at the frozen surface, and increasing ice crystal size with sample depth (Figure 7). The increase in ice crystal size with depth is quasi-linear because the heat transfer efficiency decreased with depth. This relation was previously observed on gelatine samples of similar dimensions and for a similar freezing rate (Woinet, Andrieu, & Laurent, 1998).

#### 3.2. Thermal influence on ice crystal size

It is well known that the size of ice crystals is linked to the thermal kinetics. In the literature, the most interesting local thermal parameter is the characteristic freezing time (Bevilacqua et al., 1979). It can be defined as the time necessary to change the temperature from the initial freezing point ( $-0.7^{\circ}\text{C}$  for our gel) to the temperature where 80% of the water is converted to ice ( $-10.4^{\circ}\text{C}$  for our gel) at a given point in the system. Another interesting parameter, but to a lesser degree, is the thermal gradient at the phase change interface (Faydi et al., 2001). Using the model developed in the companion paper, we performed a numerical simulation corresponding to this experimental condition and calculated these two parameters from the evolution of the local temperature.

Ice crystal size, obtained by X-ray micro-tomography on the eight sub-VOI, is plotted against the characteristic freezing time (Figure 8a) and the thermal gradient at the phase change interface (Figure 8b). As expected, ice crystal size increases with the characteristic freezing time (Bevilacqua et

al., 1979). The smaller ice crystals are obtained for larger thermal gradients at the phase change interface, as presented by (Faydi et al., 2001).

The good agreement between these results obtained with X-ray micro-tomography, in association with the numerical simulations, and the previous studies showed that this method is appropriate to determine the impact of experimental parameters on ice crystal size. A similar procedure has recently been applied satisfactorily to green asparagus stems (Ando et al., 2019).

### 3.3. Impact of microwave irradiation on ice crystal size

#### 3.3.1. Impact of irradiation time at constant power

First, we set the microwave incident power at 1 W, applied continuously and by pulses of 30 s periods. Several duty cycles were studied (Table 1); from 0 (conventional freezing without microwaves) to 1 (continuously applied microwaves). The mean equivalent diameter of ice crystals for the different modalities are presented in (Figure 9a).

For a constant incident power of 1 W, ice crystal size decreased significantly for longer duty cycles. It is noteworthy that the reduction is quasi-linear for low duty cycles (to 0.5) (Figure 9b). For higher duty cycles (0.75 and 1), the decrease in ice crystal size is no longer significant ( $p > 0.05$ ) with the increase of the duty ratio. For these two modalities, ice crystal size was decreased by 25% in comparison with conventional freezing (duty cycle = 0).

These results are particularly interesting because they show a clear decrease in ice crystal size thanks to microwave assistance. Furthermore, the fact that the smallest ice crystals were found for continuously applied microwaves, which do not induce temperature oscillations, tends to invalidate the hypothesis according to which local temperature fluctuations due to microwave pulses affect crystallisation and are responsible for ice crystal size reduction.

X-ray micro-tomography gave more information on the ice crystals because the size distributions were also available (Figure 10). We can see that the ice crystal size distributions for the smaller duty ratios are close to the reference (conventional freezing) whereas the three other modalities show smaller crystals, and a narrower distribution. This trend is confirmed by the normal law standard deviation  $\sigma$  (Figure 11). The larger the standard deviation, the wider the distribution:

$$f(d) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{d-\mu}{\sigma}\right)^2} \quad (1)$$

*With  $d$  being the diameter,  $\sigma$  the standard deviation, and  $\mu$  the mean diameter.*

For duty cycles of 0.4, 0.5 and 0.6 at constant microwave power, Xanthakis et al. (Xanthakis et al., 2014) found a reduction in ice crystal size in comparison with conventional freezing although the reduction was significant only for a duty cycle of 0.6. Hafezparast-moadab et al. (Hafezparast-

moadab et al., 2018) also found a significant reduction in ice crystal size for radiofrequency (27.12 MHz) assisted freezing, but no significant difference was found for several duty ratios (0.33, 0.5 and 0.6). The smallest duty ratio used in the current study (0.167), which tends to show a reduction in ice crystal size, is smaller than those studied in the literature until now. Nonetheless, there is no data corroborating that a small duty ratio, lower than 0.33, has an impact on ice crystal size. Lower efficiency in decreasing ice crystal size could be due to an over-long period without microwave irradiation or insufficient energy supplied to the system, because the results presented here and in the previous studies are for a constant incident microwave power with different duty cycles. To determine the parameter responsible for ice crystal size distribution, additional experiments were performed at a constant energy supplied to the system.

### 3.3.2. Impact of duty ratio at constant energy supplied to the system

To study the impact of irradiation time on ice crystal size reduction, certain experiments were carried out at different duty cycles with the same energy supplied to the system (Table 2).

The mean ice crystal diameter did not evolve significantly for the different duty cycles when the same amount of energy was supplied to the system (Figure 12). We observed a mean equivalent diameter slightly larger for the smallest duty cycle (0.167), but this difference did not fall outside the standard deviation. However, the ice crystal size distribution was wider for the duty cycle of 0.167 whereas the duty cycles of 0.33, 0.5, and 1 did not present a significant difference ( $p > 0.05$ ) in distribution width (Figure 13a). This observation is confirmed by plotting the normal law standard deviation against the duty ratio (Figure 13b). For the same amount of energy supplied to the system, this difference could be explained by a longer time between two microwave pulses or by an insufficient pulse time. The shortest pulse time in previous studies (Anese et al., 2012; Hafezparast-moadab et al., 2018; Xanthakis et al., 2014) was 10 s whereas in our study, it was 5 s. Likewise, the time between two electromagnetic wave pulses in studies showing a significant impact on ice crystal size (Anese et al., 2012; Hafezparast-moadab et al., 2018; Xanthakis et al., 2014) did not exceed 20 s. In the current study, the time between two pulses for a duty ratio of 0.167 was 25 s. During this time, part of the sample could freeze quickly without being exposed to microwaves during the phase change, and so without perturbation to crystallisation. This could result in the slightly larger mean equivalent diameter of crystals and a wider distribution. The time between two pulses could thus be a more important factor than the duty cycle. In the current study, the period was kept constant, but a shorter period could be more appropriate for smaller duty ratios, by reducing the time of non-exposure to microwaves between two pulses.

## 4. Conclusion

This experimental study was performed on a laboratory scale prototype specifically designed to investigate the impact of microwave assistance on ice crystal sizes during the freezing of a methylcellulose gel. The ice crystal sizes were measured by X-ray micro-tomography after a freeze-drying stage. This protocol was first validated by obtaining the expected measurement of ice crystal distribution during conventional unidirectional freezing. Several microwave assisted freezing modalities were studied. The experiments were conducted with pulsed and continuously applied microwaves. The impact of microwave irradiation time was studied at a constant incident microwave power of 1 W, and then at a constant energy of 30 J supplied to the system for periods of 30 s. We observed a significant reduction of up to 25% in ice crystal size when microwave assistance was applied during freezing for both pulsed and continuously applied microwaves. These observations tend to disconfirm the hypothesis according to which ice crystal size reduction is due to temperature oscillations. Therefore, the only remaining hypothesis is the one which assumes that the reason for ice crystal size reduction is the perturbation of the H-bond network in water, which could be a precursor of the crystalline structure. It appeared that at a constant incident microwave power, the increase in irradiation time tended to decrease ice crystal size. At constant energy supplied to the system, no significant difference in ice crystal size reduction was observed except for the smaller duty ratio, which could be due to an over long time of non-irradiation between two pulses. The amount of energy supplied by the microwaves seems to be a factor influencing ice crystal size. Since all the experiments were performed with the same duration, it was not possible to confirm the reduction in ice crystal size distribution width with the increase in duty ratio. The influence of exposure time and non-exposure time on microwave irradiation will be investigated for different durations in future work.

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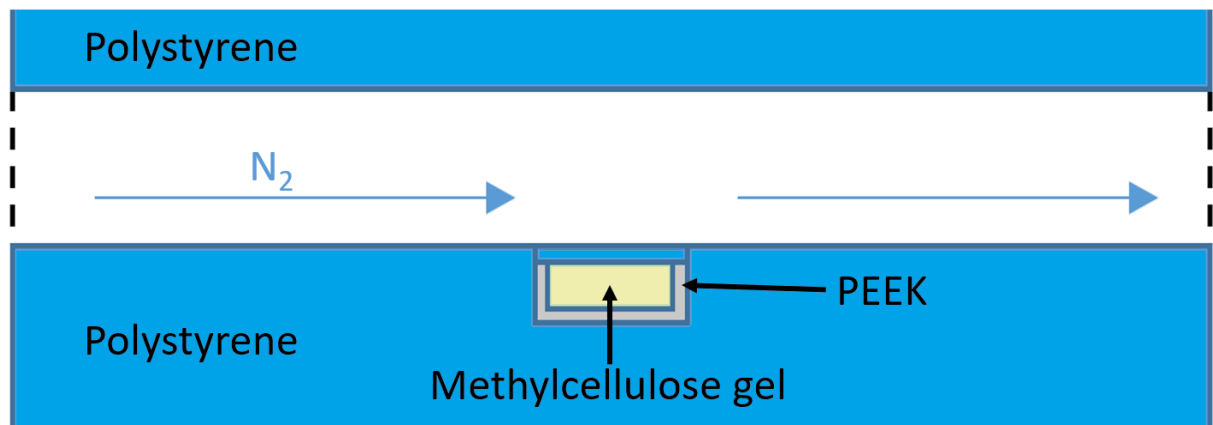
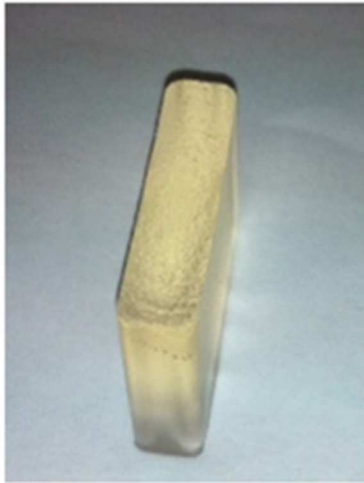
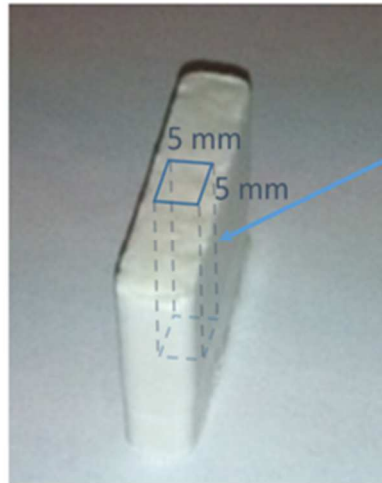


Figure 1: Experimental configuration to study microwave assistance impact on ice crystal size.

a)



b)



c)

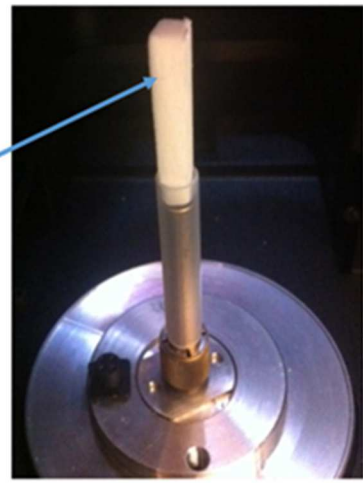
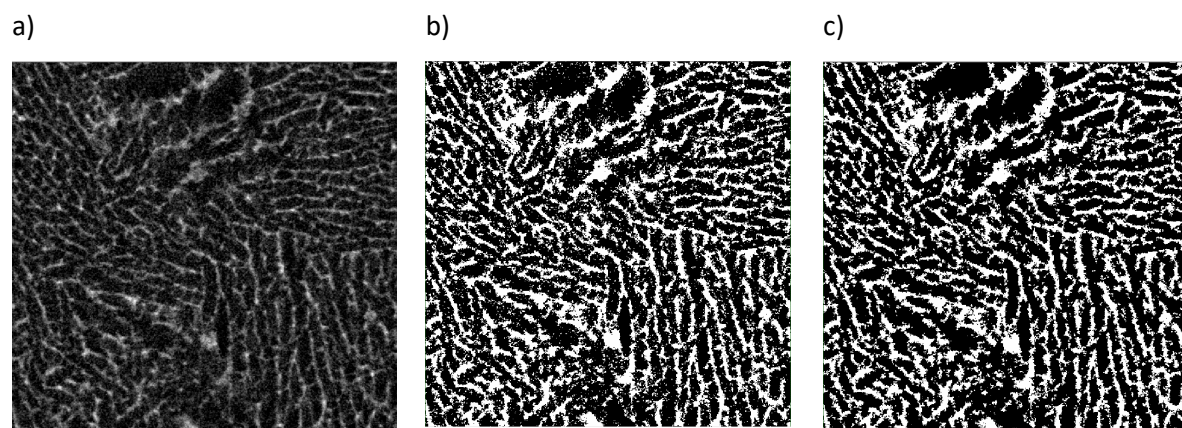


Figure 2 : a) Fresh methylcellulose gel (height: 25 mm) b) freeze-dried sample after freezing c) cut sample in the tomograph.



*Figure 3: Slice of a freeze-dried sample a) after reconstruction b) after binarisation c) after aberrant pixel removed.*

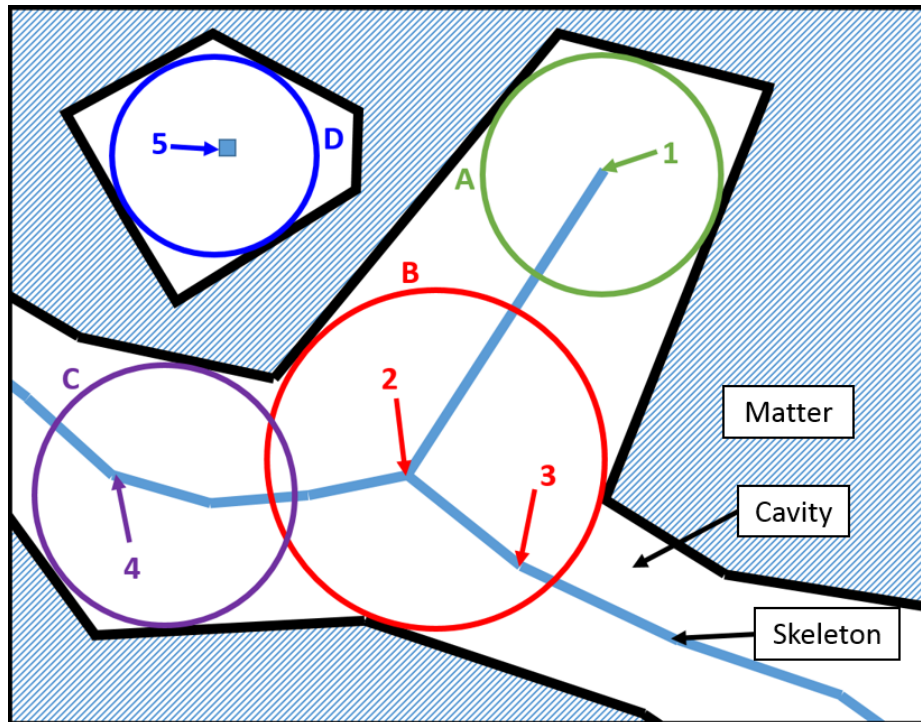
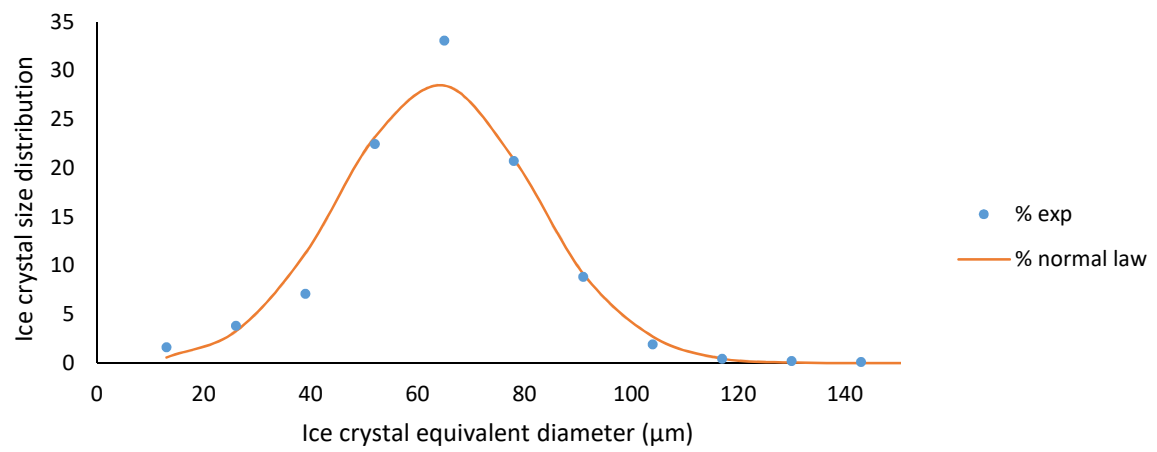


Figure 4: Illustration of the ice crystal equivalent diameter measurement principle.

a)



b)

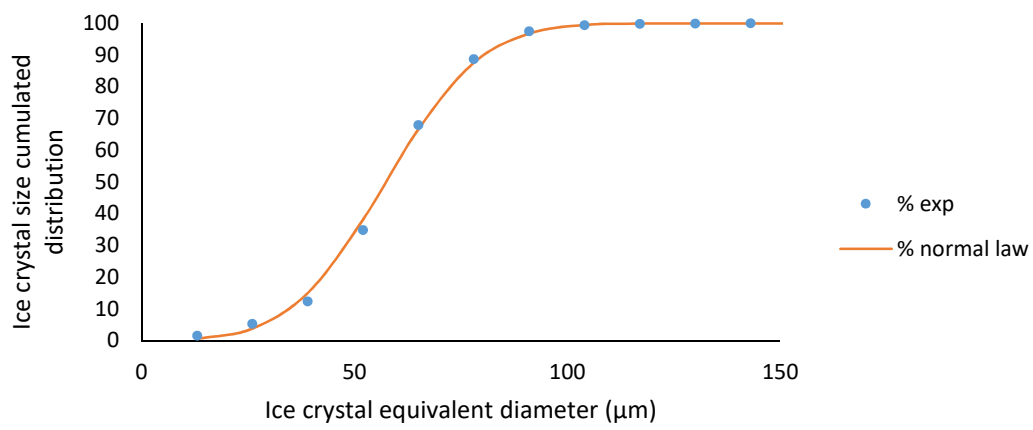
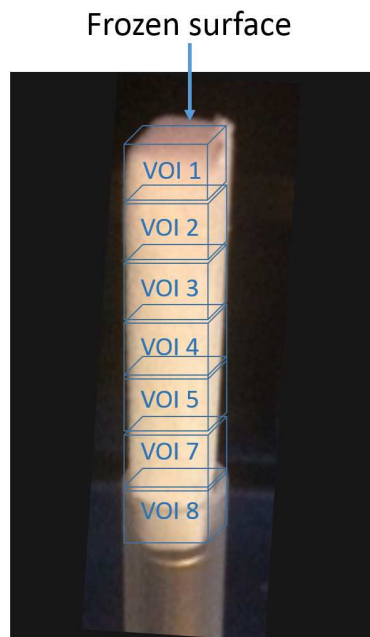
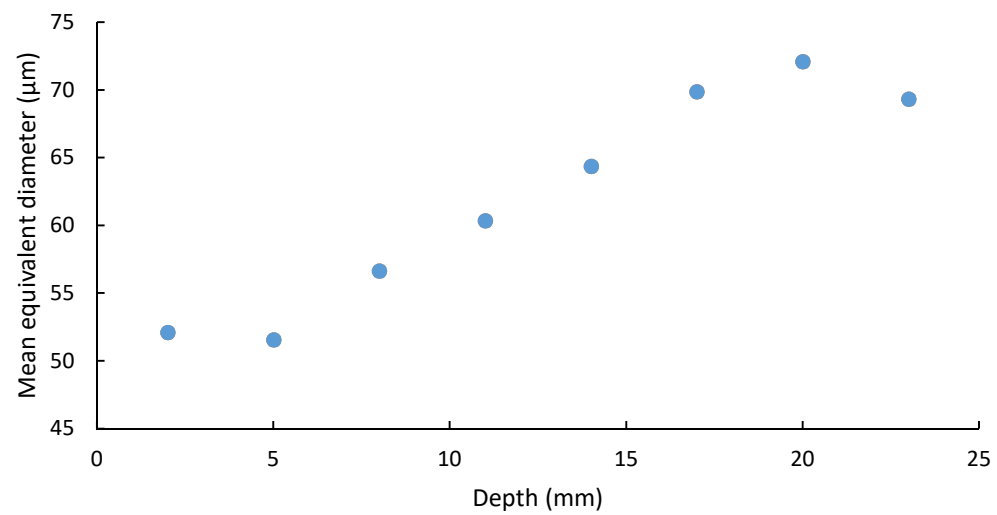


Figure 5: a) Sample global ice crystal size distribution b) Sample global cumulative ice crystal distribution.



*Figure 6: Sub-VOI locations.*

a)



b)

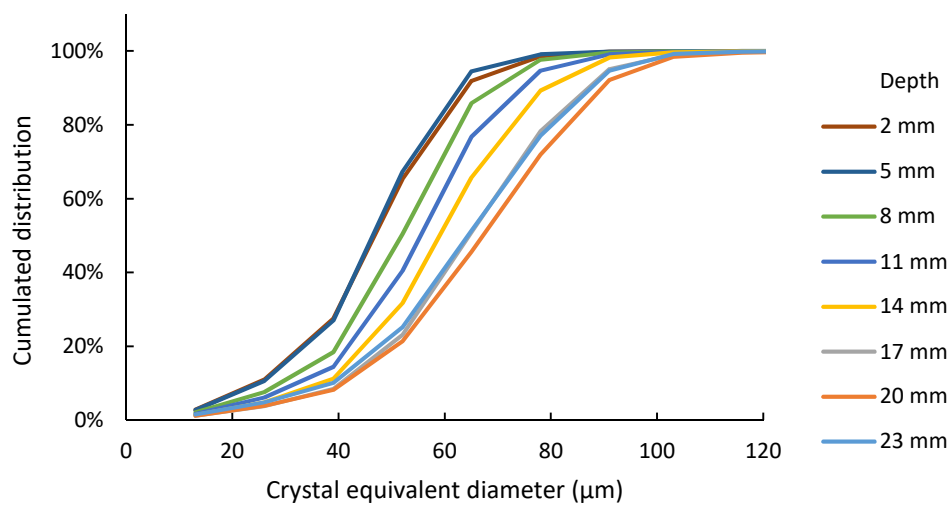
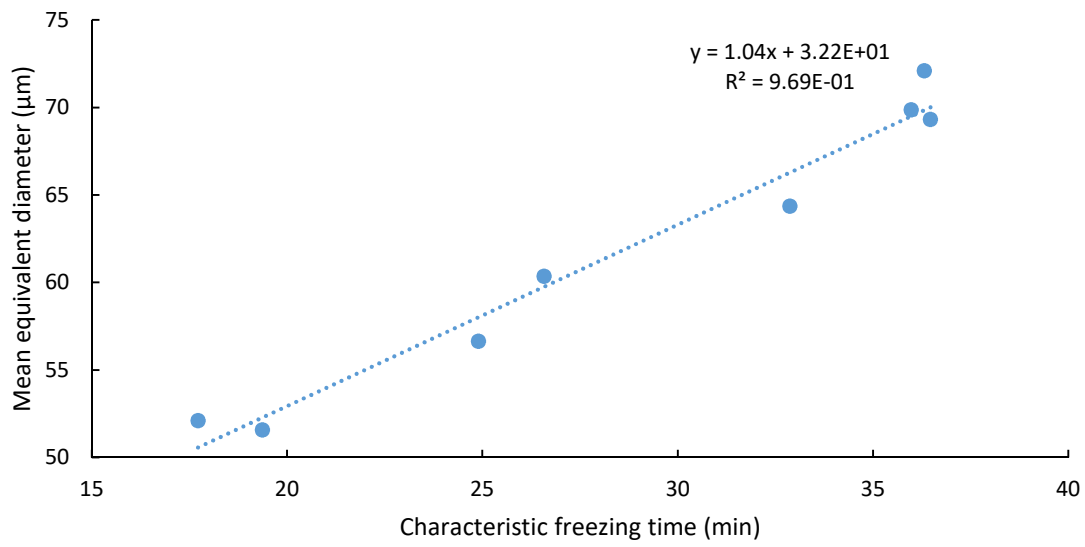


Figure 7: a) Ice crystal size as a function of sample depth b) cumulated distribution of ice crystal size at 8 different depths.

a)



b)

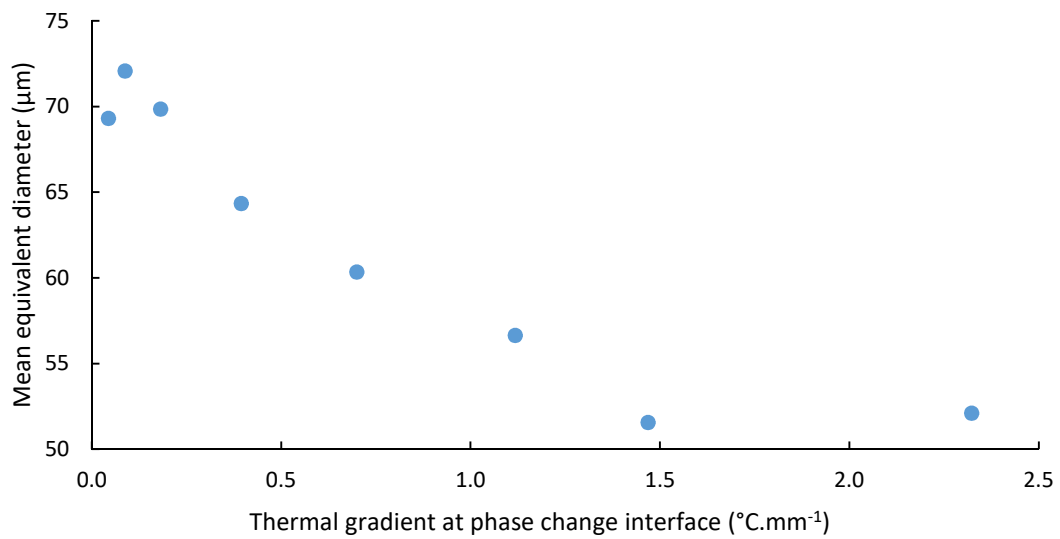
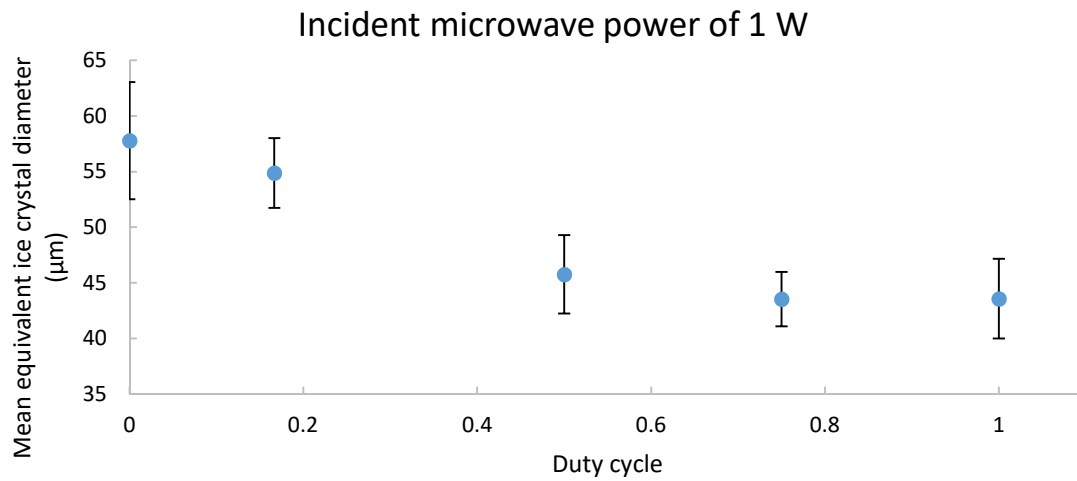


Figure 8: Mean equivalent diameter of ice crystals as a function of a) characteristic freezing time; b) thermal gradient at phase change interface.

a)



b)

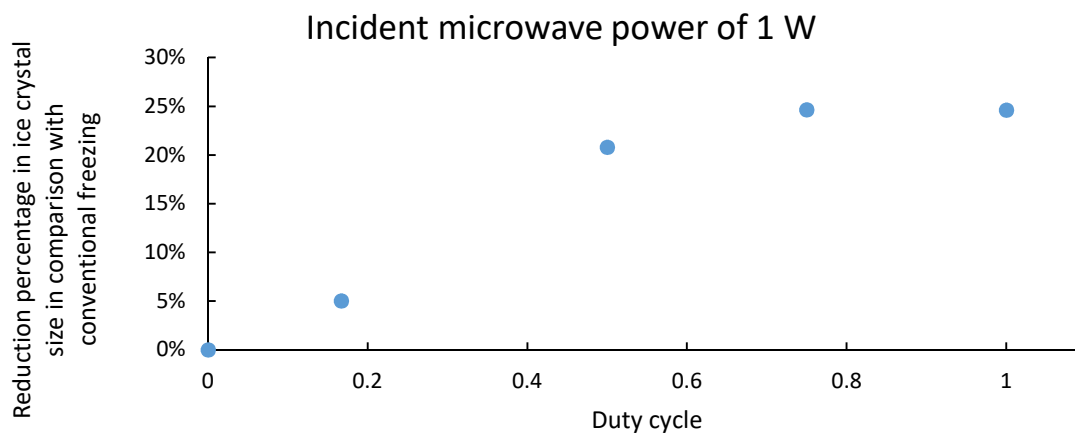
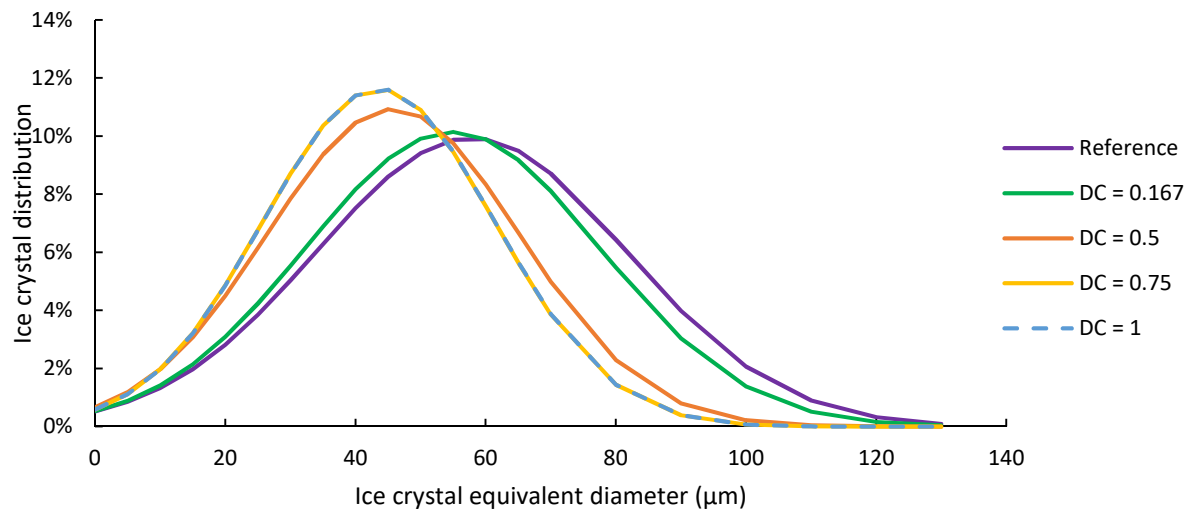


Figure 9: a) Mean equivalent diameter of ice crystals (error bars represent the standard deviation); b) reduction percentage in comparison to conventional freezing; as function of duty ratios 0, 0.167, 0.5, 0.75 and 1, corresponding respectively to 0 J, 5 J, 15 J, 22,5 J and 30 J for 30 s of freezing.

a)



b)

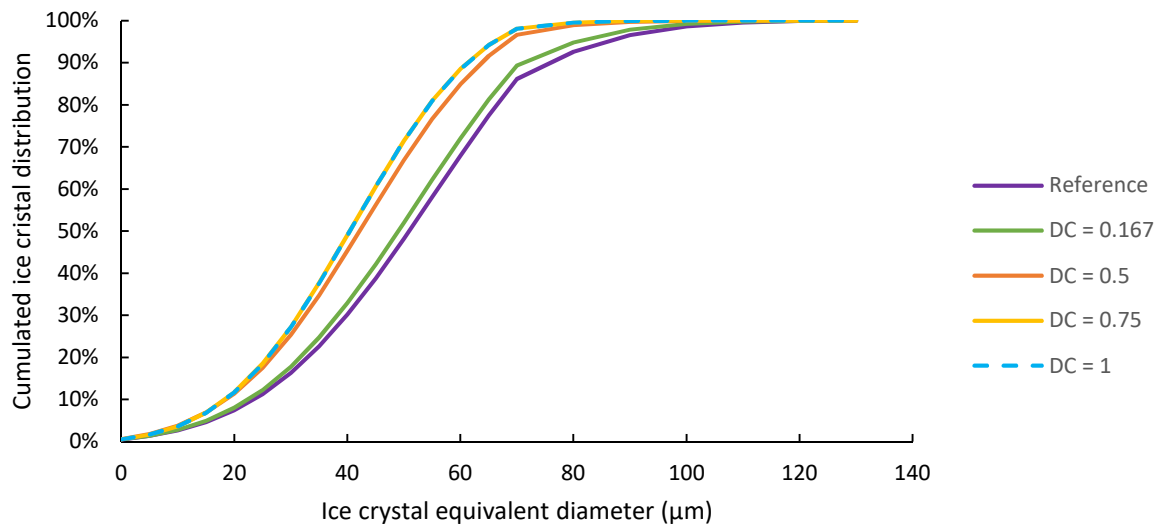
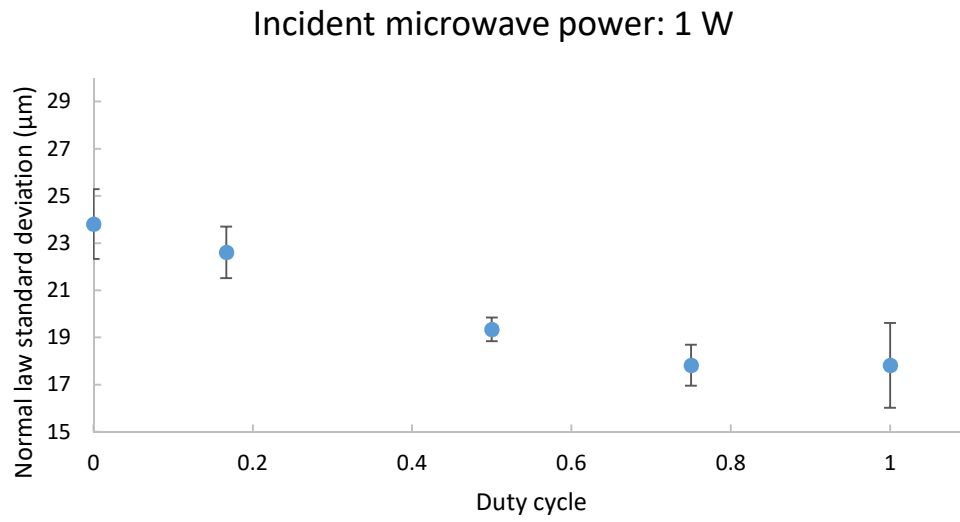


Figure 10: a) Distribution; b) cumulated distribution; of ice crystal size for different duty cycles at an incident microwave power of 1W.



*Figure 11: Normal law standard deviation describing the ice crystal distribution width as a function of duty cycle (error bars represent the standard deviation).*

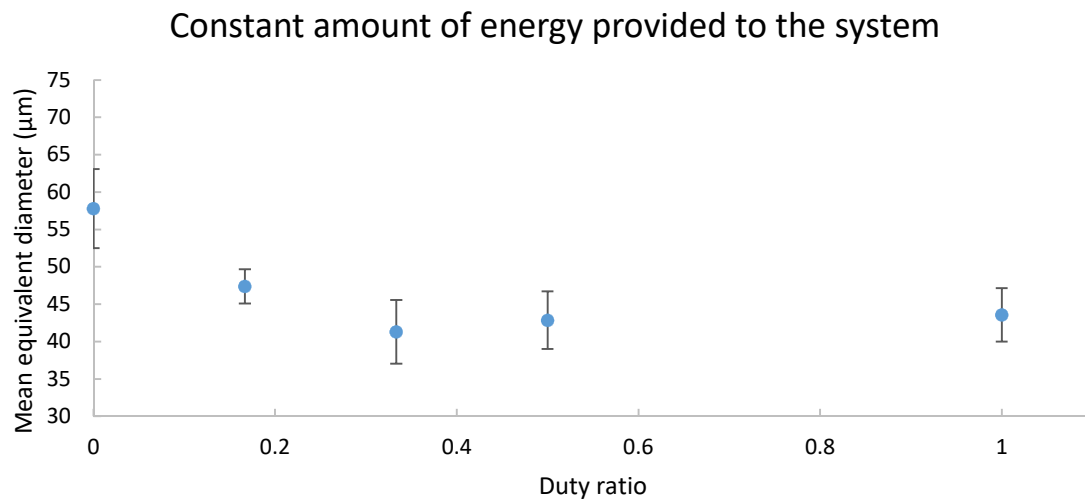
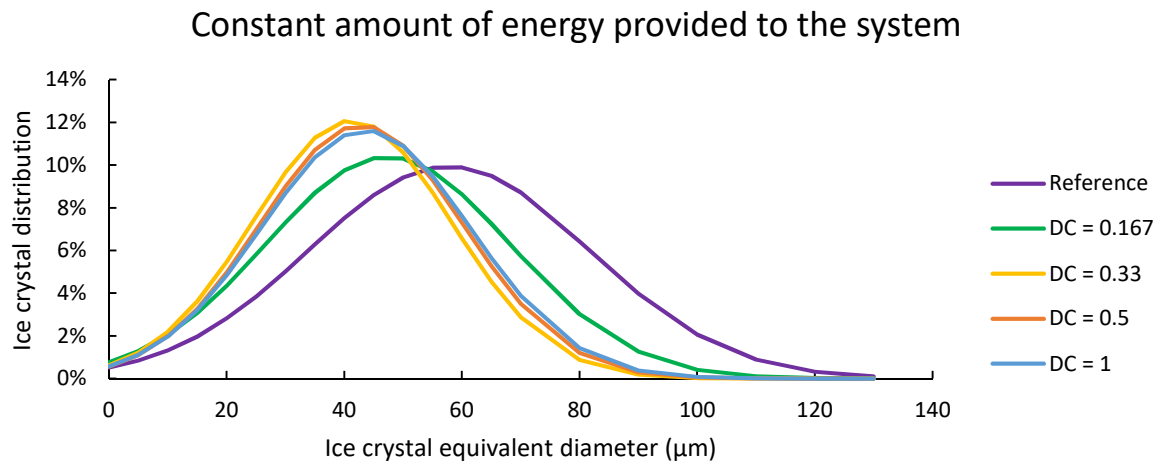


Figure 12: Mean equivalent diameter as a function of duty cycle for a constant energy provided to the system (error bars represent the standard deviation).

a)



b)

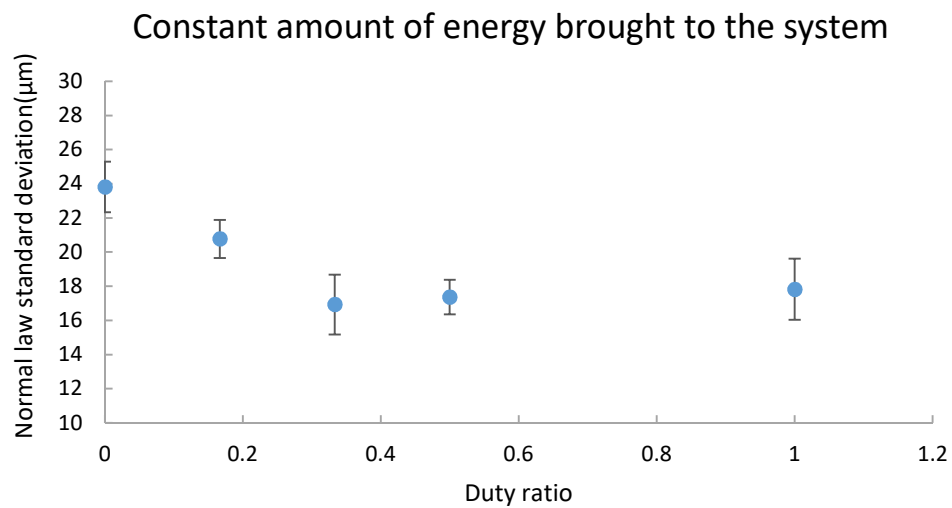


Figure 13: a) Ice crystal size distribution for different duty cycles at constant energy provided to the system b) normal law standard deviation describing the distribution width as a function of duty ratios (error bars represent the standard deviation).

Table 1: Microwave assisted freezing modalities for constant microwave power (1 W).

<b>Time (s)</b>	<b>With microwaves</b>	0	5	15	22.5	30
	<b>Without microwaves</b>	30	25	15	7.5	0
<b>Duty cycle</b>		0	0.1667	0.5	0.75	1

Table 2: Microwave assisted freezing modalities for constant energy brought to the system.

<b>Microwave power (W)</b>	1	2	3	6
<b>Duty cycle</b>	1	0.5	0.333	0.167
<b>Energy brought to the system in 30 s (J)</b>	30	30	30	30