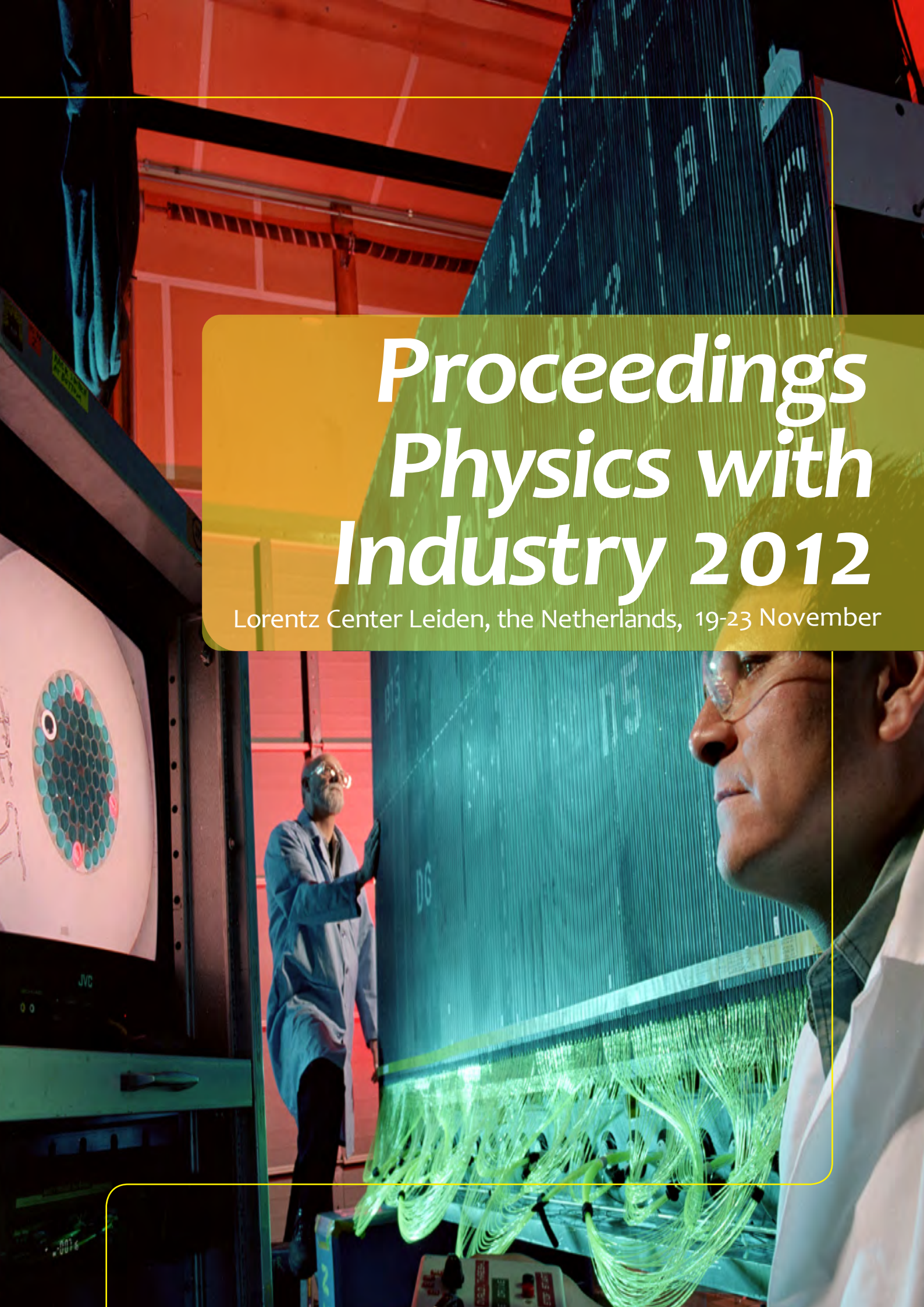

# Proceedings Physics with Industry 2012

Lorentz Center Leiden, the Netherlands, 19-23 November

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# Colophon

**Text**

Participants and organization workshop.

**Cover photo**

'Physicists assemble pieces of the ATLAS detector for the Large Hadron Collider'.
Image courtesy Argonne National Laboratory.

**Design and production**

Stichting FOM, Utrecht, the Netherlands.

**Contact**

Isabel Poyck
Foundation FOM
PO Box 3021
3502 GA Utrecht
Phone: +31 (0)30 600 12 21
Email: isabel.poyck@fom.nl

**Thanks to**

The organisation is particularly grateful for the excellent service and facilities of the Lorentz Center, the effort of the senior researchers (from the preparation phase onwards) and the enthusiastic contribution of all participants during the week.

The workshop 'Physics with Industry' was organised by Marcel Bartels and Pieter de Witte of the Foundation for Fundamental Research on Matter (FOM) and Floor Paauw and Marjan Fretz of the Technology Foundation STW in collaboration with Sietske Kroon of the Lorentz Center. The event was funded by the Lorentz Center (which is partly funded by FOM and STW) and the participating companies. FOM is part of the Netherlands Organisation for Scientific Research (NWO). STW is funded by NWO and the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

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# Foreword

It is our great pleasure to present to you the proceedings of the third 'Physics with Industry' workshop that was organised by the Foundation FOM and Technology Foundation STW at the Lorentz Center in the Netherlands. The main aim of the 'Physics with Industry workshop' is to obtain creative solutions for industrial problems and to bring (young) physicists in contact with industrial R&D.

The first 'Physics with Industry workshop' was organized in 2010 and was inspired by the 'Mathematics with Industry' workshops, which have regularly been organised by the 'International Study Group Mathematics with Industry' since 1968. As well as enabling excellent scientific research, both FOM and STW focus on contributing to the Dutch knowledge economy, for example through public-private research collaborations and the training of young scientists. The 'Physics with Industry workshop' is therefore a natural extension of FOM's and STW's ambition to help companies and to inspire (young) physicists.

59 scientists participated in the workshop 2012, ranging from PhD students to professors. These scientists spent a week working in groups on five industrial problems, which were selected by a programme committee from proposals put forward by industry. Following an introduction to the various problems by the companies on Monday, the participants worked on these in groups for the rest of the week. On Friday, the groups presented their findings to the companies.

Besides the scientific outcomes, the workshop also resulted in new public private contacts that may lead to future collaborations. A novelty in 2012 was that one company (NXP) filed a patent based on the results of this workshop. Participants were mostly driven by the shear pleasure of applying their physics knowledge to new problems, the desire to enrich their scientific network and the interest in gaining hands on experience with industrial R&D processes. Companies benefited from the scientific input they received and participating in the workshop enlarged their academic network.

These proceedings provide an overview of the scientific results obtained during the third 'Physics with Industry' workshop. We hope you enjoy reading it!

Wim van Saarloos
Director FOM

Eppo Bruins
Director STW

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# Introduction

The third workshop Physics with Industry was organized in 2012 by the Foundation FOM and Technology Foundation STW at the Lorentz Center at Leiden, the Netherlands.

The five industrial problems discussed during the week were collected via an open call for proposals in spring 2012. A programme committee selected the five 'best problems' for the workshop. The selection criteria used by the committee were:

- it must be possible to solve the problems (or a major solution must be within reach) within one week and physics can make a clear contribution to the solution;
- it should be an urgent problem;
- the company should be willing to share detailed information.

The committee aimed at a mix of contributions from small, medium and large companies. The committee consists of seven researchers with different backgrounds in physics:

Prof. Marileen Dogterom, FOM Institute AMOLF
Prof. Ute Ebert, Centrum Wiskunde & Informatica
Prof. Erik van der Giessen, University of Groningen
Prof. Fred MacKintosh, VU Amsterdam
Dr. Jacco Snoeijer, Twente University
Dr. Peter Steeneken, NXP Semiconductors
Prof. Lucas van Vliet, Delft University of Technology

The committee selected problems from the companies Janssen Precision Engineering, Microdish, NXP (Leuven), PamGene and Shell. Together a well balanced mix of SME's and larger industries posing different challenges ranging from fundamental questions to more applied problems in a wide variety of industries. As soon as the five workshop problems had been selected, senior researchers from academia who are familiar with the specific subjects involved were recruited. They helped the companies to prepare their questions for the workshop and they joined the workshop week to guide the progress of the discussions.

These proceedings contain five chapters, one for each company case. Each chapter starts with a description of the case and a profile of the company, followed by a detailed description of the results obtained in the single workshop week.

PamGene International

# PamFreezer: a solution to enable frozen biopsy logistics

Bram Colijn[1], Gonzalo Ríos Cruellas[1], Maryam Hashemi-Shabestari[2], Xander Janssen[3], Theo van der Leij[4], Roy Leyte-Gonzalez[5], Srinivas Vanapalli[1], Pim Veldhuizen[6], Gerard Verbiest[2], Bram Verhaagen[1]

[1] University of Twente, the Netherlands
[2] Leiden University, the Netherlands
[3] Technical University Delft, the Netherlands
[4] PamGene International BV, the Netherlands
[5] Eindhoven University of Technology, the Netherlands
[6] Utrecht University, the Netherlands

## Abstract

Tissue samples that are taken during a biopsy need to be snap-frozen in order to preserve their properties and use the tissue for contemporary molecular biology technologies that may improve the treatment of the patient. There is currently a lack of (safe) methodologies or devices for snap-freezing tissue. Furthermore, there is a lack of knowledge on the optimal cooling rate, which depends on the type of tissue and is important to know in order to avoid damage to the cells.
This report comments on the biological background of the acceptable cooling rates and also describes a design for a new biopsy snap-freezing device. The suggested device fulfills the requirements for use inside a hospital environment. The device consists of a cooling unit and a base station. The copper cooling unit can be pre-cooled on the base station until used. After biopsy, the tissue sample inside a cryovial can be deposited into the cooling unit and is then cooled down at rates between 1-10 K/sec, which is within the biologically safe range for several tissue types. The cooling unit may then be transported for several hours while keeping the tissue sample below 193 K.

# 1. Company profile

PamGene was founded in December 1999 and is a company engaged in the research, development, manufacturing and commercialisation of life science applications based on its proprietary micro array technology.
PamGene is a biomarker company, focusing on opening up new opportunities for the development of Personalized Medicine (pharmaceutical discovery, translational and clinical). Most of PamGene's projects are in oncology and many of these involve medicines that inhibit or modulate cellular kinases and kinase pathways as well as nuclear receptors and their signaling mechanisms. Our collaborators apply our technologies for biomarker research and to support drug discovery in areas including tumor tissue and compound profiling in diseases concerning kinases in Oncology and also in several other diseases and fields of expertise such as the Central Nervous System, Immunology and Obesity.

# 2. Problem description

Access to frozen biopsies is of tremendous importance to improve the treatment choices for patients. To enable contemporary molecular biology technologies, such as kinase activity testing, fresh/frozen tissue is a prerequisite. The problem is that frozen patient material (biopsy) is not seen as a routine source for diagnostic testing in clinical practice. Current practice in a hospital does not include a simple and reliable solution for this yet.
When cancer is suspected, a variety of biopsy techniques can be applied. One type of surgery, called a core needle biopsy, uses a large, fitted needle to extract a sample of tissue about the size of a piece of pencil lead. A core needle biopsy can take place in a clinic or hospital and it can be performed by e.g. an internist, radiologist, or surgeon. Current practice in a hospital uses chemical fixation (formalin) directly after tissue acquisition to preserve tissue from degradation, and to maintain the structure of the cells. The latter is needed as a definite diagnosis of cancer is almost always based on the histological examination of tissue samples.

To enable contemporary molecular technologies fresh/frozen tissue is a prerequisite. Fresh tissue is obtained by means of snap freezing for which solid carbon dioxide, liquid nitrogen ($N_2^{liq}$) or isopentane cooled with liquid nitrogen are used as coolants to keep good morphology of the tissue and to keep the molecular activity intact. Snap freezing tissues for diagnostic and research purposes are therefore often time consuming, laborious, even hazardous and not user friendly and are therefore not applied at the location of biopsy acquisition.

A simple device is foreseen which facilitates easy and reliable logistics of frozen patient material from the location where the biopsy is removed to the pathology laboratory where the biopsy is examined and archived.
This device is composed out of two parts:

- a special designed micro-tube facilitating easy removal of the tissue from the sample notch of the biopsy needle (the PamTainer)
- a cryogenic device enabling temporary freezing of the tissue collected in the micro-tube (the PamFreezer).

In this workshop we focus on the PamFreezer design. The PamFreezer should be able to replace the currently used coolants while snap freezing the tissue directly after its acquisition, to allow temporary storage and to bridge the transportation time of the frozen tissue to the pathology laboratory.

Main characteristics of the device include:

- Fast cooling ratio (cooling well below 273 K in seconds)
  Note: details of the cooling rate as well as the required lowest cool temperature are not known though literature shows sufficient results at cooling times of 10 - 30 seconds either at $N_2^{liq}$ or dry ice. Cooling in a 193 K fridge however, is not allowed.
- The sample should remain below 193 K for at least one hour, during transport from the place of biopsy taking to the storage/analysis location somewhere else in the hospital.
- Preferably only electricity is allowed as utility, alternatively $CO_2$ cartridges may be used (no safety vessel).
- Low weight, user friendly, limited servicing required, low noise, etc.
- Autoclavable.

### 2.1 Geometry

The biopsy tissue sample is taken with a 8G needle (largest case), which has an inner diameter of 3 mm. The length of the notch in this needle is 1 cm (average case); its volume is then 70 $mm^3$. A cylindrical geometry of the sample is assumed. The type of tissue, and therefore also the (thermal) properties, may vary, however that variation is typically within a few percent. The cryovial in which the sample is placed has typical dimensions of 10 mm outer diameter, 5 cm in length and a wall thickness of 1.5 mm, and is made of polypropylene.

### 2.2 Energy estimates

For the "back of an envelope" estimate of the demanded cooling power, the following assumptions are used:

- Tissue sample is initially at body temperature (310 K).
- Vial is initially at room temperature (293 K).
- The vial and sample have to be cooled down to 77 K within 10 seconds.
- The specific heat of the materials is temperature dependent.
- As the specific heat decreases and is in literature often only specified at specific temperatures, linear interpolation is used as the trend is linear for most materials in first order approximation.

Using these assumptions and the material properties listed in table 1, an estimate can be made for the amount of heat to be extracted.
The heat to be extracted from the tissue sample is given by:

$$E_{tissue}\ (T = 310\ K \rightarrow 273\ K) = \rho V c_p \Delta T = 1 \cdot 0.07 \cdot 4 \cdot 37 = 10.4\ J$$

$$E_{phase\ transition}\ (T = 273\ K) = \rho V L = 1 \cdot 0.07 \cdot 334 = 23.4\ J$$

$$E_{tissue}\ (T = 273\ K \rightarrow 77\ K) = \rho V c_p \Delta T = 1 \cdot 0.07 \cdot 2 \cdot 193 = 27.0\ J$$

Total amount of heat from the tissue: 60.8 J

| Biological tissue [1] | |
|---|---|
| Density ($\rho$) | 1 g/cm$^3$ |
| Heat capacity ($c_p$) T > 273 K | 4 J/g·K |
| Heat capacity ($c_p$) T < 273 K | 2 J/g·K |
| Thermal conductivity ($k$) T = 293 K | 0.5 W/m·K |
| Latent heat ($L$) | 334 J/g |
| | |
| Cryovial (polypropylene) [2] | |
| Density ($\rho$) | 0.85-0.95 g/cm$^3$ |
| Heat capacity ($c_p$) T = 293 K | 1.5 J/g·K |
| Heat capacity ($c_p$) T = 77 K | 0.5 J/g·K |
| Thermal conductivity ($k$) T =293 K | 0.16 W/m·K |
| | |
| Copper | |
| Density ($\rho$) | 8.96 g/cm$^3$ |
| Heat capacity ($c_p$) T = 293 K | 0.4 J/g·K |
| Heat capacity ($c_p$) T = 77 K | 0.2 J/g·K |
| Thermal conductivity ($k$) T =293 K | 400 W/m·K |
| Thermal conductivity ($k$) T =77 K | 557 W/m·K |

**Table 1.** Material properties of the biopsy tissue sample, the cryovial and copper

with $c_p$ the temperature dependent specific heat, $\Delta T$ the change in temperature, $\rho$ the density and $V$ the volume of the sample. The latent heat $L$ is required for the phase-transition from liquid to solid around the freezing point of water (second line of calculation).

The heat to be extracted from the vial is calculated in a similar way by:

$$E_{vial}\ (T = 310\ K \rightarrow 77\ K) = m_{polypropylene} c_p \Delta T = 1 \cdot 1 \cdot 233 = 233.0\ J$$
$$E_{air\ in\ vial}\ (T = 310\ K \rightarrow 77\ K) = m_{air} c_p \Delta T = 0.001 \cdot 1 \cdot 230 = 0.2\ J$$

Total amount of heat from the vial: 233.2 J

with $c_p$ the temperature dependent specific heat, $\Delta T$ the change in temperature and $m$ the mass of the vial material (typically 1 g for a 3 mL vial).

Total amount of heat from the vial and the tissue sample: $\approx$ 294 J

In order to cool the vial and sample to the desired temperature of 77 K within 10 seconds, a cooling power of 29 W is needed:

$$P_{desired} = \frac{total\ energy}{cooling\ time} = \frac{294\ J}{10\ s} \approx 29\ J/s = 29\ W.$$

Note that the amount of heat to be withdrawn and hence the cooling power, increases linearly with the volume of the biological material and/or the size of the vial.
As small and low-noise cryogenic coolers (suitable for application in a hospital environment e.g. pulse-tube or Stirling) have a typical cooling power of 10 W, the demanded cooling rate of the sample cannot be achieved directly. As a solution, we suggest to use a "cold reservoir" that is pre-cooled to a low temperature (e.g. 77 K), in which the sample is inserted and hence cooled down rapidly.
As the cold reservoir absorbs the heat from the sample and vial, the temperature of this reservoir will increase inevitably. To calculate the temperature increase of such a reservoir made out of copper, we use the following assumptions:

- The cold reservoir is at cryogenic temperature (77 K).
- The cold reservoir is perfectly insulated from the environment, i.e. heat flux to surrounding is zero.
- The heat from the tissue sample and vial is fully transferred to the cold reservoir.
- The specific heat of the cold reservoir is temperature dependent.
- As the specific heat decreases and is in literature often only specified at specific temperature, linear interpolation is used as the trend is linear for most materials in first order approximation.
- The mass of the copper block is 232 grams.

The increase in temperature of the cold reservoir upon taking all the heat from the sample and vial is given by:

$$\Delta T = \frac{E_{uptaken}}{c_p m} = \frac{291}{0.3 \cdot 232} = 4\ K$$

with $\Delta T$ the change in temperature, $E_{uptaken}$ the energy to be withdrawn from the sample and the vial, $c_p$ the temperature dependent specific heat of the cold reservoir and $m$ the mass of the cold reservoir. So a block of 232 grams of copper cooled down to 77 K will heat up to 81 K when the vial and the sample are inserted and have obtained a thermal equilibrium with the cold reservoir.

# 3. Benchmark: biopsy in cryovial in $N_2^{liq}$

The current procedure for biopsy snap-freezing (submersion of the cryovial in liquid nitrogen [$N_2^{liq}$]) was evaluated as benchmark case. No literature on the temperature evolution of the tissue sample during this procedure is known. On the other hand, protocols are not standardized across hospitals or operators. Commonly used protocols include submersion of the tissue in $N_2^{liq}$ for a varying number of seconds. A Leidenfrost effect (thermal shielding by vaporization of $N_2^{liq}$ near the surface) may occur, which may be related to the 'hissing' sounds that are reported. To prevent this, cycles of submersion into and extraction from $N_2^{liq}$ are also reported to be used, which may reduce the cooling rate significantly. However, details on the temperatures involved are missing.
We have investigated numerically the temperature evolution of a piece of tissue inside a vial, which is immersed in a liquid nitrogen bath at 77 K.

## 3.1 Numerical model

A one-dimensional numerical model was constructed, which assumed a piece of tissue ($T_t$ = 310 K, thickness of 1.5 mm) resting against a polypropylene wall ($T_w$ = 298 K, thickness of 1.5 mm), with liquid nitrogen ($T_n$ = 77 K) on the other side of the wall.
Inside the tissue, the wall and the liquid nitrogen, the one-dimensional heat (diffusion) equation is solved:

$$k\frac{\partial^2 T}{\partial x^2} = \frac{\partial T}{\partial t} \tag{1}$$

where $x$ is the distance from the center of the tissue outward.

At the boundary between the vial wall and the liquid nitrogen, heat transfer is modeled:

$$Q = -hA\frac{\partial T}{\partial x} \tag{2}$$

The heat transfer coefficient $h$ is not known exactly for these two materials, but for liquid nitrogen with steel this number is around 250 W/m$^2$·K [3] and we assume it to be 100 times smaller for plastic (which is based on the ratio of heat conduction of plastics and steel). When there is a thin layer of vaporized nitrogen near the wall (Leidenfrost effect), $h$ is reduced by another factor of 6 (which is based on the ratio of heat conduction of nitrogen in liquid and vapor phase). All thermal properties of nitrogen in liquid and gas phase are obtained from the NIST database [4]. The thermal properties of the tissue and the vial are listed in table 1.
At large $x$ the temperature of the liquid nitrogen is assumed constant at 77 K. At $x = 0$ there is a symmetry boundary condition.

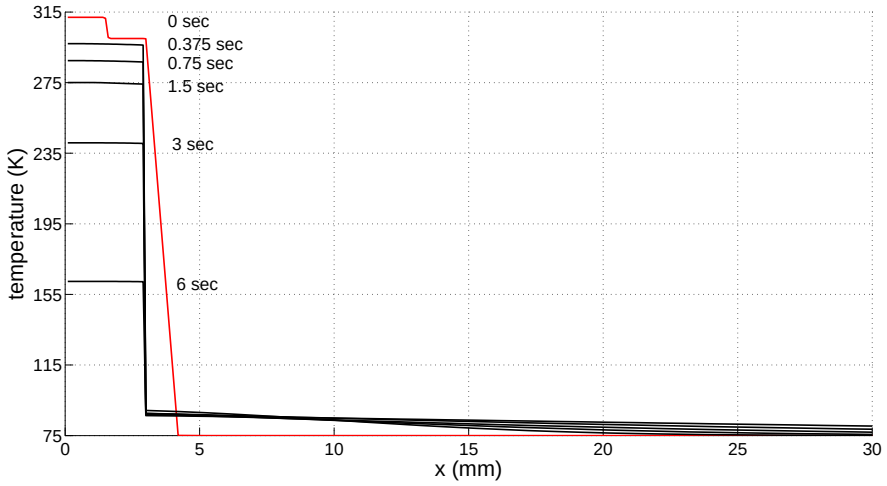


*Figure 1.* Temperature profiles at 6 selected time steps, showing the cooling of a tissue sample ($x = 0 - 1.5$ mm) next to a wall ($x = 1.5 - 3$ mm) immersed in liquid nitrogen ($x = 3 - 30$ mm).

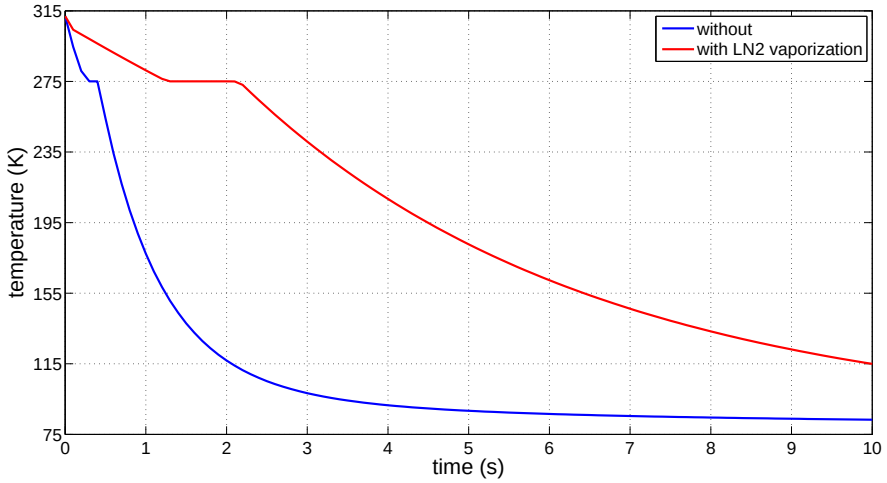


*Figure 2.* Temperature evolution of the center of the tissue sample as a function of time. A nitrogen vapor layer formed on the vial surface leads to significant thermal shielding and therefore a reduced cooling rate.

Equations 1 and 2 are solved numerically using explicit finite-difference discretization, with spatial steps of 0.1 mm and temporal steps of $10^{-5}$ s.
The phase change of the tissue when freezing near 273 K is incorporated by setting the heat capacity to 334 W/K when the temperature of the tissue is between 272.5 and 273.5 K.

### 3.2 Results

Figures 1 and 2 show that the temperature of the tissue sample decreases rapidly towards zero, where there is a phase change, before the temperature decreases rapidly towards 193 K and below. A nitrogen vapor layer near the vial wall leads to significant thermal shielding that slows down the cooling of the tissue sample by a factor of 6.
The tissue sample reaches a temperature of 193 K within 5 seconds (including $N_2^{liq}$ vaporization), with cooling rates around 40 K/sec. This cooling rate is likely to be overestimated for several reasons, e.g. the heat transfer across the several interfaces may not be so perfect as assumed here. Nevertheless, the model gives an order-of-magnitude estimation of the cooling of the tissue sample and of the important factors for this cooling.

## 4. Cooling rate limits

It is generally thought that snap-freezing should involve cooling rates as high as possible, in order to preserve the tissue as good as possible. Determining the exact limits for the cooling rates for the new snap freezing device require a more careful look at the underlying biological phenomena, which will be done in this section.

### 4.1 Biological aspects influencing the cooling rate

Liquid water is essential in both function and structure of living cells. Freezing water can be lethal for the cells and paradoxically can also preserve cells for long periods of time in a viable state, and it permits the long-term storage of tissues and organs. Depending on the rate of freezing or thawing, the sub-cellular constituents and the details of cell structure can be preserved or become disrupted. Thus the rate of freezing and thawing of the cells is very

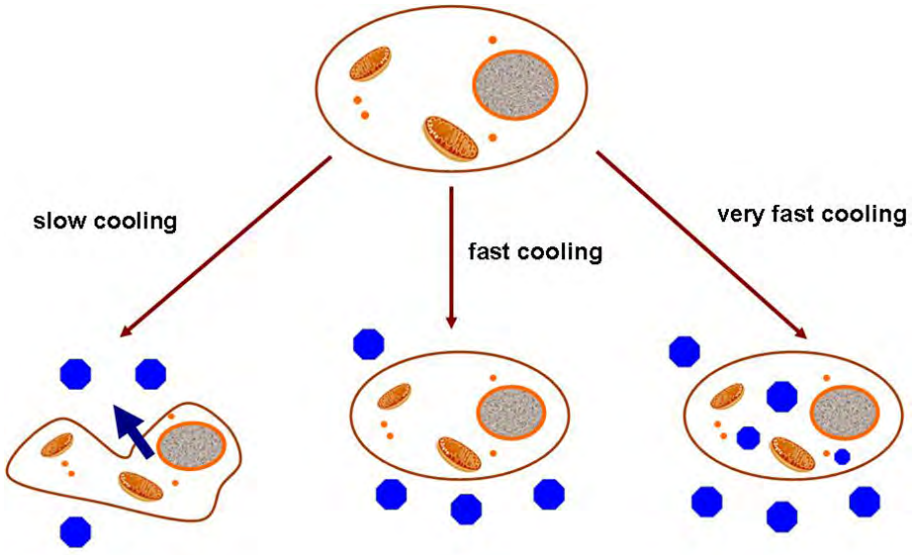


*Figure 3.* Schematic presentation of crystal formation in cells.

critical. As demonstrated cells are subjected to chemical, thermal and mechanical forces during cryopreservation, which can profoundly affect their biological function [5].
It has been observed that during freezing the intracellular ice formation induces significant damage to the cells. Two main damage mechanisms have been proposed during freezing. At slow freezing, the chemical potential difference across the cell membrane (as a result of extracellular ice formation) may lead to cell dehydration. The dehydration can cause significant cell damage [6]. Additionally extracellular ice formation can cause significant thermo-mechanical stress leading to mechanical cell damage (figure 3).
The cooling rate will determine the size of the crystals, and depending on the size of the crystals the damage to the cells will be in a higher or lower degree. Therefore, the speed of cooling down the tissue plays an important role in cryopreservation. To design our cooling device, it is essential to take the optimum rate of cooling/freezing into account.

### 4.2 Method for estimating the optimal cooling rate

The rate of intracellular ice nucleation depends on the type of cells. The key parameters are permeability of the cell membrane to water ($L_{pg}$), apparent activation energy ($E_{Lp}$), and the ratio of the available surface area for water transport to the initial volume of intracellular water ($SA/WV$).
The optimized cooling rate $B_{opt}$ (K/minute) can be determined using the following equation [7]:

$$B_{opt} = 1009.5e^{-0.0546E_{Lp}}L_{pg}\frac{SA}{WV} \tag{3}$$

The $B_{opt}$ values for several types of cells have been calculated, see table 2. These values show that the cooling rate for different types of cells including tumor cells can be different. To have the maximum survival of cells we need to define the optimum cooling rate range (figure 4). Depending on the cell type, this optimal cooling rate range can be different.
Toner and coworkers developed a model from which they calculated the intracellular nucleation rate [8]. Later Karlsson *et al.* improved Toner's model [5]:

$$J(T) = \Omega e^{-\kappa T^{-3}\Delta T^{-2}} \tag{4}$$

Here $J(T)$ is the nucleation rate of the intracellular ice formation in a given cell population undergoing cryopreservation, $\Omega$ is the kinetic coefficient, $\kappa$ is the thermodynamic coefficient, and $\Delta T$ is the supercooling. The $\Omega$ and $\kappa$ coefficients are different for each cell type. Toner *et al.* used mouse oocyte. Based on the $\Omega$ and $\kappa$ coefficients of the mouse oocyte they suggested an optimum cooling range of 60 to 600 K/minute (or 1 to 10 K/sec). We used this range further to design our apparatus.

## 5. PamFreezer design

Here we present a design idea (figure 5) for a PamFreezer cooling unit to snap freeze the biopsy tissue sample, which also includes a PamFreezer base station. The design is based on

| cell type | $L_{pg}$ | $E_{Lp}$ | $SA/WV$ | % error | $B_{opt}$ (K/sec) |
|---|---|---|---|---|---|
| lymphocyte | 0.10 | 15.5 | 0.74 | 5.3 | 0.5 |
| tumor cells | 2.71 | 55.4 | 0.3 | 31 | 0.7 |
| spermotosoma | 0.01 | 29.2 | 11.1 | 10 | 0.3 |
| oocyte | 0.04 | 13.3 | 0.10 | 5 | 0.03 |

**Table 2.** Calculated values of $B_{opt}$ for several types of cells using the values report in [7].

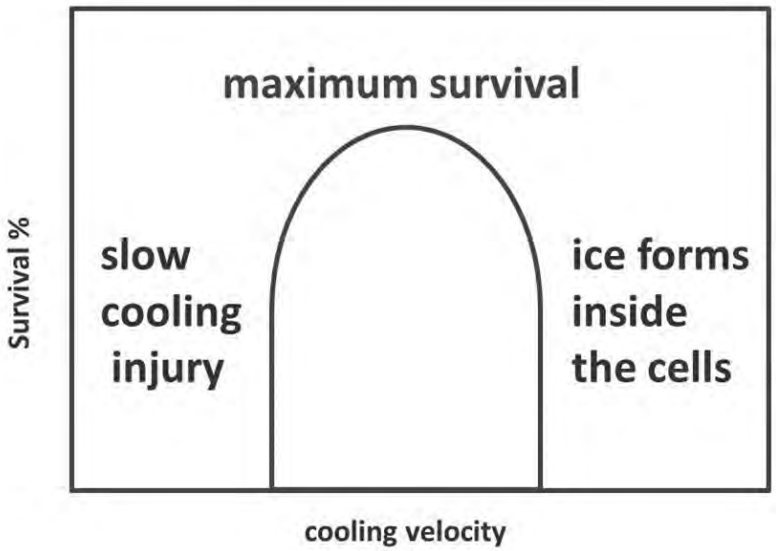


*Figure 4.* Schematic presentation of maximum cell survival depending on the cooling rate.

the cooling rates and thermal conditions stated above.

### 5.1 Cooling unit

The central piece of the device is a cylindrical copper block of 232 grams weight, 1.75 cm radius and 3.25 cm height. It has a chamber in the centre to place the cryovial with the tissue sample inside, and an extra piece of copper on the top that acts as a cover. The cooling unit is pre-cooled in the base station (described below) until it needs to be used.
Copper was chosen because of its good thermal conductivity and high heat capacity. Furthermore, it is cheap (ca. € 6,-/kg). The total mass of copper was calculated to fulfill its two main functions: as a "cooler" and as a "cold maintainer" inside the cryovial. For the first function, the block is estimated to be pre-cooled to 77 K in about 30 minutes. The second function is to absorb external heat once the block is separated from the cooler, thereby

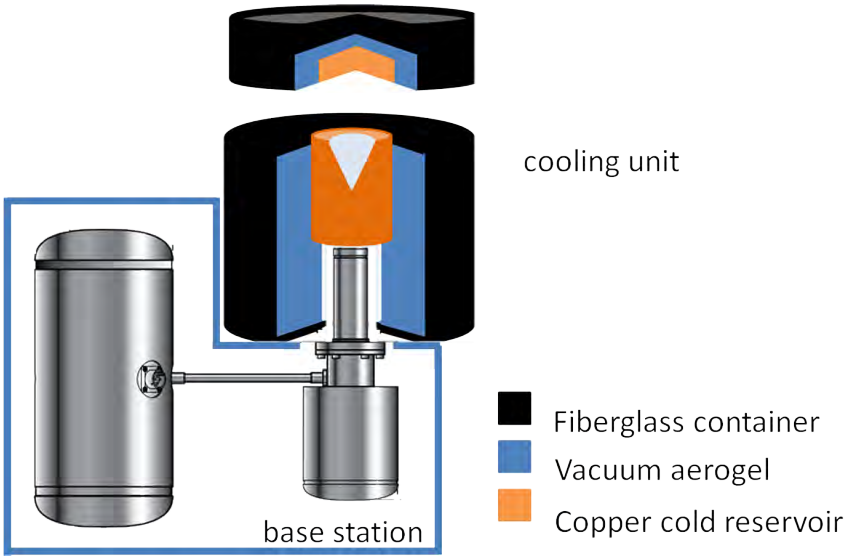


*Figure 5.* Sketch of the PamFreezer design.

cushioning the temperature increase of the cryovial.
The cooling unit is light in order to be transported through the hospital for at least one hour to the storage/analysis location.

### 5.2 Cryovial chamber

The cryovial and the cryovial chamber both have a conical shape, which allows for pushing the cryovial into the chamber to ensure maximum contact. A soft plastic for the cryovial material may enhance the fitting of the vial in the chamber. Additionally (or alternatively), the wall of the chamber may be covered with a thermal conduction material that enhances the contact between wall and vial, such as Velbond [11].

### 5.3 Insulating cover

In order to ensure that the temperature of the biological sample stays below 193 K for 1 hour without active cooling, the insulation of the cold reservoir and its mass i.e. heat capacity have to be taken into account. As the mass of the block is limited for practical reasons to lower than 0.5 kg, the insulation that is used is critical. First choice (table 3) could be low-density polystyrene as it is low cost and easy to manufacture/shape. However, as the device has to be heat-sterilized (typical temperatures 393 - 473 K), polystyrene cannot be used (melting point of 373 K). A better candidate would be a ridged container made from e.g. fiber glass (already widely used in non-magnetic cryostats) filled with e.g. glass wool or Perlite.
A recently developed material, aerogel has proven to be a very good insulator with the extra benefit of having an extremely low density. It is thus an ultralight material suitable for insulating, due mainly to its low thermal conductivity (30 mW/m·K), which can be even further enhanced by introducing a vacuum (or low pressure). Furthermore, the material is relatively cheap (ca. € 20,- for one cooling unit). In the PamFreezer, the function of an aerogel cover is to insulate the copper block, in order to prevent heat transfer from the ambient. Except the part of the bottom wall that is in contact with the cooler, all the surface of the copper block should be covered by at least 5 cm of aerogel.
In addition, the cold part of the cooler is also covered with aerogel to reduce heat gains from the surroundings. An extra piece of aerogel with the same thickness is reserved to replace the cryocooler once the device is ready for transport.

### 5.4 Base station: cooler selection

The base station is a fixed device that can cool down the cooling unit from room temperature to 77 K, and compensates the heat absorbed from the surroundings until the cooling unit needs to be used. The main component of the base station is a cryocooler.
There are some requirements for the cooler selection that should be satisfied, like the cooling power and the minimum temperature attainable. The copper is cooled down from room temperature to 77 K in 30 minutes, so the required cooling power is 5 W. Moreover, since the

| Material | Thermal conductivity (mW/m·K) @ T = 293K |
|---|---|
| Air (w/o convection) | 24 |
| Polystyrene | 30 |
| Fiberglass | 40 |
| Glass wool | 40 |
| Aerogel @ 1 atm | 30 |
| Aerogel @ 0.1 atm | 3.2 |
| Perlite @ 1 atm | 31 |
| Perlite @ “vacuum” | 1.4 |

**Table 3.** Thermal conductivity of several insulation materials

device is intended for use in hospitals, it should meet certain requirements for facility of use and safety reasons. To satisfy the requirements of the biopsy taking workplace, the device should not have vibrating parts to avoid excessive noise.
Initially, thermoelectric (Peltier) devices were considered as it is easy to control, cheap and has a low level of noise. However, the temperature limit they can reach is 193 K, which is not enough for our purpose.
The second option was the Stirling cooler, capable to reach cryogenic temperatures with powers up to 15 W. Although they work with helium as the working fluid, they satisfy the safety requirements since they are sealed systems and are therefore without any possible contact of the operator with hazardous fluids. Reasons against this cooler are its weight, price and noise. However, the cooling device is intended to remain in the surgery room, hence the weight is not a significant problem.
The last option is pulse tube cryocoolers, that also operate with helium and are capable to reach cryogenic temperatures. In contrast with Stirling, this cryocooler can be operated without moving parts (less noise) in the low temperature side of the device, making the cooler suitable for our application. Pulse tube cryocoolers are available from Qdrive (USA), Sunpower (USA), Thales Cryogenics (Europe), AIM (Europe), Honeywell Hymatic (UK) and Ricor (Israel) at prices in the k€ range.

## 5.5 Usage protocol

The PamFreezer has a cooling down time of approximately 30 minutes and may be (auto-)switched on during the night so that it is ready for operation at 8 AM.
The biopsy is removed from the biopsy needle and placed inside the cryovial, preferably making as much contact with the cryovial as possible. The cryovial is then gently pushed inside the cryovial chamber of the PamFreezer cooling unit. The insulation cap is then added to the PamFreezer cooling unit. After one minute, the PamFreezer cooling unit may be undocked from the PamFreezer base station, and the bottom insulation should be added to the PamFreezer cooling unit. Once the PamFreezer cooling unit is undocked, the cooling unit starts to warm up and there is a time limit of 1 hour before the temperature of the tissue sample has increased to 193 K.
After returning to the location where the biopsies are taken, the PamFreezer cooling unit can be re-docked on the PamFreezer base station after removing the bottom insulation and replacing the cooling cap (which has a cryovial chamber notch that prevents condensation of air on the chamber surface). The PamFreezer cooling unit is then cooled down (from room temperature) within 30 minutes.

## 5.6 Additional device considerations

### Biopsy sample inside the cryovial

The heat transport between the biopsy tissue sample and the cryovial is affected by the positioning of the sample inside the vial. The heat transport encounters a resistance at each interface, however this is very much material-dependent and is not easily obtained from the literature. The heat transport increases linearly with contact area (equation 2). Ideally, the sample would fill the entire lowest part of the conical cryovial.

### Auto-switch on/off

The operator of the PamFreezer may be assisted by including an auto-switch in the base station design, that detects the docking of the PamFreezer cooling unit (using an electrical contact or pressure sensor) and switches the base station off once the cooling unit is undocked.

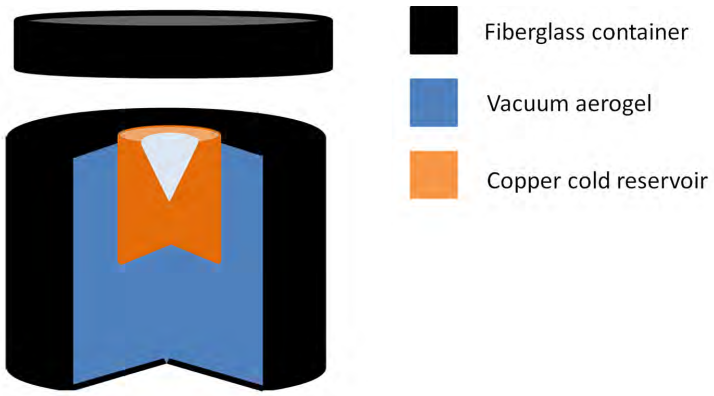


*Figure 6.* Sketch of the budget solution for cooling down the cooling unit.

### 5.7 Budget solution

As currently available cryocoolers are expensive (k€ range), we envision a solution that is based on using liquid nitrogen. Instead of using it close to the patient (not desired), it may be used in a central facility in a hospital. By carefully choosing the mass of the copper block (1 kg) and the insulation (5 cm thick vacuum aerogel; thermal conductivity 0.001 W/m·K, heat capacity 0.84 J/g·K and density 1 kg/m$^3$), the temperature of the cold reservoir increases only from 77 K to 100 K within 10 hours. After cooling down this cooling unit in the central facility using $N_2^{liq}$, it can be transported to and stored in the area where it is used for snap-freezing. After snap-freezing, the tissue sample can stay in the device for another few hours as long as the device with sample is transported to the final destination within 10 hours after pre-cooling. Although it still requires on $N_2^{liq}$, this solution can be used in a pilot study for the applicability of cooling biological tissues using a solid material (e.g. copper).

## 6. Simulations of the PamFreezer cooling device

The performance of the proposed PamFreezer design is tested using finite element analysis executed with COMSOL Multiphysics software. A two-dimensional axisymmetric model is employed to calculate the heat transfer by conduction during the different stages of operation of the PamFreezer. Radiative and convectional heat transfer are assumed to play no significant role. The design has three important requirements: the pre-cooling time, snap-freezing speed, and the warm up time of the biopsy tissue without cooling. Before we address these properties, we discuss the details of the finite element analysis.

### 6.1 Numerical setup

Figure 7 shows the model used in the finite element analysis. The model has a cylindrical symmetry and the symmetry axis is shown in red. We have used two configurations to calculate both the pre-cooling time and snap-freezing properties. Firstly, for the pre-cooling study we remove insulation underneath the copper and add a heat sink on the bottom boundary of the copper. And secondly, we use the configuration as shown in figure 7 for the snap freezing. COMSOL calculates automatically an appropriate number of cells, with higher mesh densities near corners and small spaces.
We have three different boundaries within the model. Firstly, we have a symmetry boundary on the axis of symmetry. Secondly, we have the outer boundaries of the isolation which are set to room temperature. And thirdly, we have a boundary between a solid and a liquid or gas, where the condition is such that the temperature is continuous across the boundary.

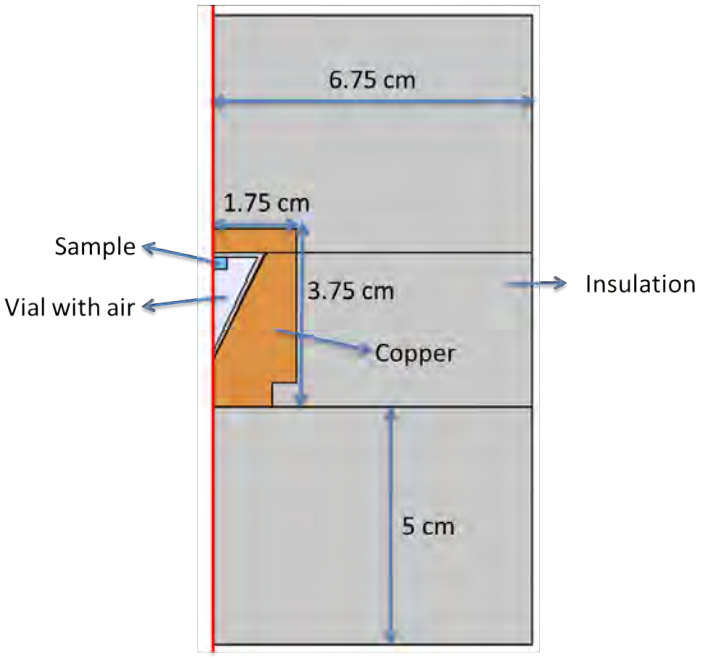


*Figure 7.* The configuration used in the finite element analysis. The red line indicates the axis of revolution.

The temperature of the insulation is always the room temperature at the start of the calculations. This is the worst case scenario for the snap-freezing and the warm up time of the biopsy tissue without cooling. In reality, the warm-up time will be larger than our result and the equilibrium temperature reached after snap-freezing will be lower than our result.

### 6.2 Pre-cooling

The pre-cooling of the copper was calculated using the cooling power characteristics of three types of commercially available cryocoolers. The three coolers have a cooling power of 2.5 W, 5 W and 10 W respectively at a temperature of 77 K. Figure 8 shows the temperature measured in the center of the copper lid, starting from the moment that the (insulated) copper is placed on the base station and the cooler is powered. The copper and insulation is assumed to be at room temperature (293 K) before cooling. As we observe, both the 5 W and 10 W cryocooler are able to cool the copper cold reservoir to 77 K within 20 minutes. The cooling rate of the 2.5 W cryocooler is significantly slower because the amount of heat leaking through the insulation is almost equivalent to the cooling power.

### 6.3 Snap-freezing

The snap-freezing of the tissue is modeled by inserting a conically shaped polypropylene vial with thickness of 1 mm and content volume of 3 mL in the pre-cooled copper. The vial contains a 0.07 mL piece of tissue that is assumed to consist purely out of water. To account for the influence of surface roughness of both the polypropylene vial and the copper cold reservoir, the vial is assumed to be surrounded by 0.1 mm of air. We also take the latent heat (23.4 J) of freezing water into account in order to model the phase transition correctly. Figure 9 shows the temperature of the center of the biopsy tissue after insertion of the cryovial into the cooling unit. The center of the tissue starts to cool down after ca. 10 seconds, as first the vial and the outer parts of the tissue have to cool down. In figure 9a we also observe that a larger temperature gradient between the copper and tissue results in a shorter snap-freeze

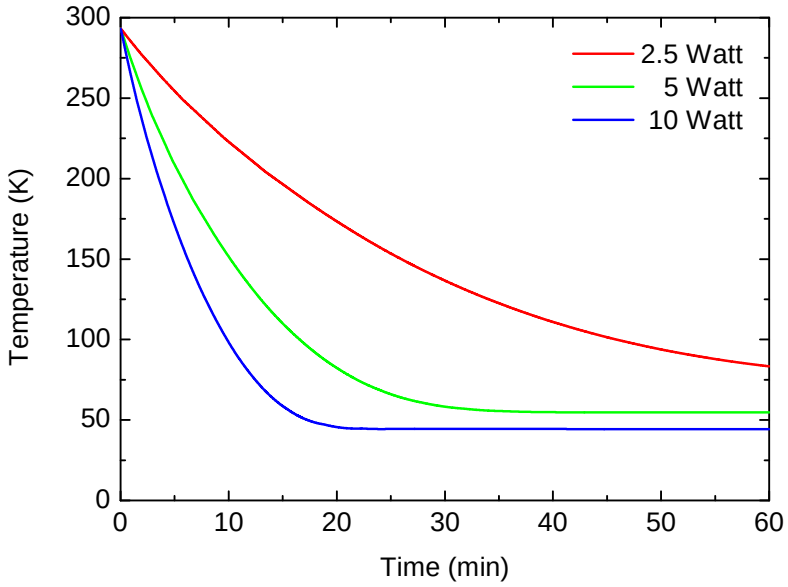


*Figure 8.* Temperature simulation of pre-cooling the copper cold reservoir using three types of cryocoolers.

time. We find a snap-freeze time of 20 s (for 77 K), 11 s (for 150 K), and 8 s (for 193 K). The cooling rates are determined at the freezing point of water and are approximately equal to, -8 K/s (for 77 K), -5 K/s (for 150 K), and -3 K/s (for 193 K), which is within the acceptable ranges as stated in section 4.
Another important observation of figure 9a is the temperature of the tissue after it reached its equilibrium temperature. We observe the equilibrium temperature to be higher than the starting temperature of the pre-cooled copper. If the pre-cooled copper is 77 K, we find an equilibrium temperature which is 23 K higher. For the other pre-cooled temperatures, 150 K and 193 K, we find that the equilibrium temperature is respectively 13 K and 10 K higher. We

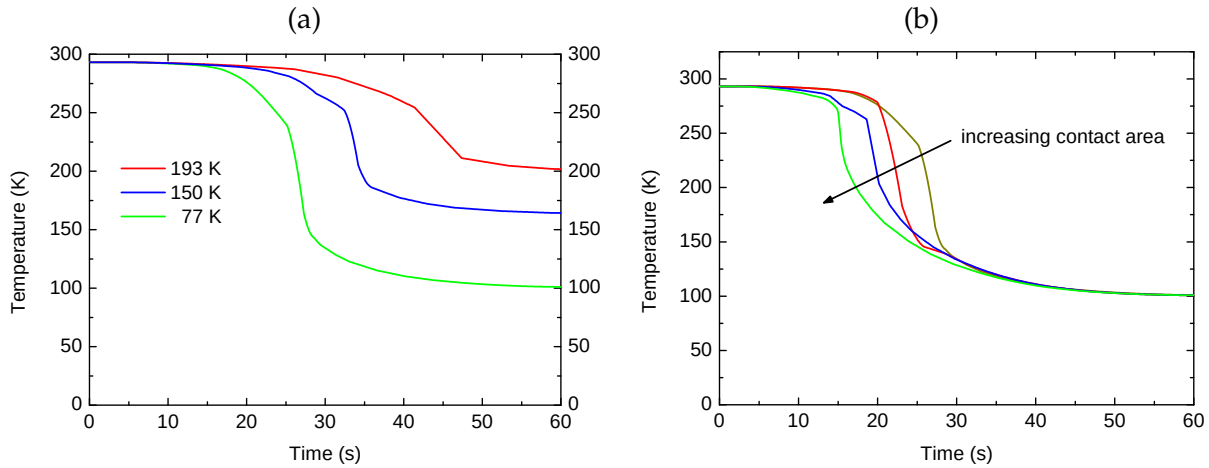


*Figure 9.* (a) Temperature as a function of time measured in the center of the biopsy tissue when inserted in a pre-cooled copper. The different colors represent the different temperatures of the pre-cooled copper: 193 K (red), 150 K (blue), or 77 K (green). (b) Temperature as a function of time while snap-freezing different tissues with the same volume but different contact areas. The temperature of the pre-cooled copper is 77 K. The contact area increases from 6.6% (olive green), to 13% (red), to 21% (blue), and to 27% (green).

attribute this difference to the heat transferred from the tissue and vial to the copper block and to the heat transferred from the insulation, which is assumed to be at room temperature, into the copper, vial, and tissue.
We assume the contact area of the biopsy tissue with the vial to be of significant importance, because heat conduction through air is worse than through the vial-tissue contact. Figure 9b shows the snap-freeze curves for different contact area. We express the contact area as a percentage of the total surface area of the biopsy tissue, which is a cylinder with constant volume of 0.07 mL. The contact area increases from 6.6% (olive green), to 13% (red), to 21% (blue), and to 27% (green). We observe that a larger contact area does not result in a faster snap-freeze time, but the snap-freezing does happen earlier in time. This indicates that the snap-freezing is limited by the thermal conduction of the tissue material and especially the latent heat necessary to freeze the tissue.

### 6.4 Warm up time of the biopsy tissue during transport

The last important issue to address is the time the sample needs to warm up to 193 K as this is the temperature at which the sample preservation is lost. For this end, we calculated the temperature within the sample an hour after snap-freezing. Figure 10 shows a typical warm up curve of a tissue snap-freezed in a pre-cooled copper block of 77 K. The temperature after one hour is 183 K, which is still below the critical 193 K. It is important to stress that there are two effects which influence this behavior. Firstly, the insulation starts at room temperature during snap freezing, thereby emptying the cool copper bath. In reality the insulation is also cooled, therefor the warm up time will be larger than an hour in reality. And secondly, the actual device will likely have a heat leak between the insulation lid and the copper, thereby possibly increasing the heat leak to the environment, this would reduce the warm up time. However, we expect that one hour warm up time is feasible within the current model.

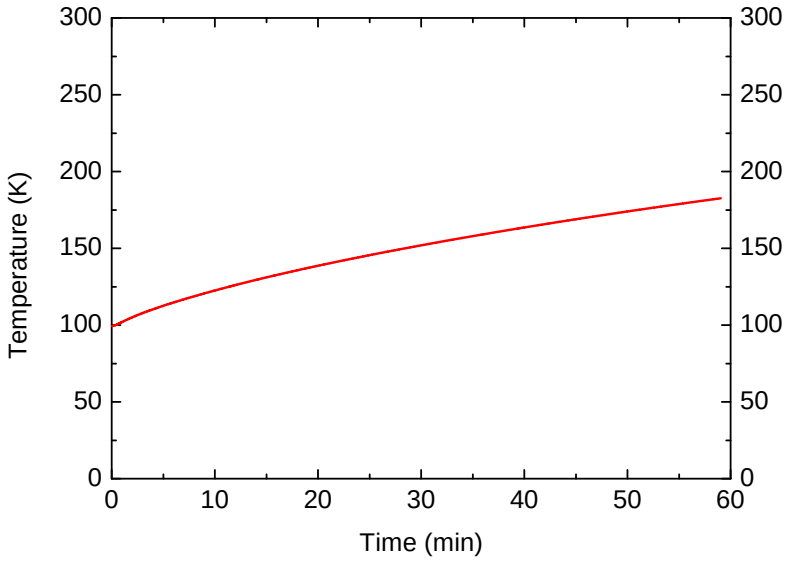


*Figure 10.* Temperature as a function of time in the biopsy tissue, which was snap-freezed in a pre-cooled copper block of 77 K.

# 7. Discussion & Outlook

The simulations show that the proposed PamFreezer design should be able to cool the tissue sample in a sufficiently fast way, while also being able to retain at low temperatures for at least one hour during transport. Furthermore, the materials for the cooling unit are cheap, however the cryocooler itself is expensive but may be replaced (initially) by liquid nitrogen.
Important next steps are the (thermal) validation of both simulations and a more thorough investigation into the optimal cooling rates.

## 7.1 Model validation

The numerical models need to be verified before their predictions can be accepted.

### Numerical validation

The numerical models for snap freezing in liquid nitrogen and in the new cooling device can be validated by comparing their typical cooling rates and characteristics. The COMSOL model furthermore needs internal verified with a grid-independency check.
The validity of the COMSOL model for the new cooling device is supported by the numerically calculated increase of the Copper block, which matches the estimation made in the Problem statement section.

### Experimental validation

The two numerical models have to be validated experimentally, by time-resolved measuring the temperature of a tissue sample during snap-freezing. No literature is known on the cooling rate of a tissue sample snap-frozen in liquid nitrogen.
Temperature measurement of a tissue sample can be done in several ways [12, 13]:

- **Thermocouple**
  A junction of two different metals may generate a voltage when heat is applied. This phenomena is used in thermocouples, which can be as small as 0.1 mm in diameter. Such a thermocouple could be placed inside the tissue sample for temperature measurements. Heat exchange between the tissue sample and the metals should be fast in order to assure that the thermocouple reads accurately.

- **Thermistor**
  The resistance of a resistor or a wire depends on its temperature, and this phenomena may be used to measure the temperature of a sample in which a wire or resistor is placed. Also for this method the wire/resistor needs to be small.

- **Thermal noise measurement**
  The amount of thermal noise on an electrical wire or component is a measure of its temperature. Proper calibration is required (also for the two methods above).

- **Thermal camera**
  Thermal cameras can record the IR radiation of objects and in that way measure the temperature of such an object. Cooled thermal cameras typically operate in the 60-100 K range; uncooled thermal cameras operate around room temperatures. The temperature resolution is generally around 2 K and these cameras are usually expensive.
  Furthermore, they only measure the temperature of the outer surface; the temperature of the core can then only be estimated using assumptions for the material properties.

### 7.2 Cooling rate

- A systematic study of optimum cooling range for different types of cells is required.
- Employment of cryomicroscopy for identification and study of ice formation (darkening of intracelullar space) is beneficial to determine the amount of damage occurred in the cells during the freezing process [9, 10].

## 8. Summary

We have proposed and simulated a design for a PamFreezer biopsy snap-freezing device, which fulfills the requirements for use inside a hospital environment. The device consists of a cooling unit and a base station. The copper cooling unit can be pre-cooled on the base station until used. After biopsy, the tissue sample inside a cryovial can be deposited into the cooling unit and is then cooled down at rates between 1-10 K/sec, which is within the biologically safe range for several tissue types. The cooling unit may then be transported for several hours while keeping the tissue sample below 193 K.
(Thermal) validation of this design is an important next step.

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