Development of a system for the investigaton of adhesion properties of nanoscopic ice layers at cryogenic temperatures

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Darmstadt, 19. September 2023

L. Widmayer

Acknowledgment

Ich danke euch

Abstract

Diese Kurzfassung ist kurz.

Contents

1 Introduction

Cells are the main building block of live. With light microscopes cells are made visible and can be studied by biologist. Many methods use fluorescence to study certain cell structures or processes in detail. For example live and dead cells are detectable with fluorescence. Still, not everything is visible even with the best light microscopes.

Light microscopy has a major limitation. The resolution of most microscopes is limited through the Abbe criterium. Many efforts are made to push and work around the limit [1]. Still, other microscopy methods are needed to see the smallest of scale. Elecron microscopy is able to resolute up to rows of Atom scale. In Biology, electron microscopy is used to see viruses and other small structures which would be otherwise invisible.

In electron microscopy, no live cells can be observed. The sample is put in a vacuum while electron microscopy. Gas would disturb the electron beam, making electron microscopy impossible. In vacuum at room temperature, Water evaporates and destroys the cell. One option is to replace the water with another substrate. The commonly used substrate is toxic, making it hard to work with. Another way is to freeze the water to ice at cryogenic temperatures. The microscope needs to be cooled to prevent the sublimation of the ice.

Light microscopy is also possible with frozen samples. Conveniently, the process of sample preparation is very similar. Still, a major difference in the preparation process exist. For electron microscopy, a thin film in combination of a mesh is used as a grid. This grid does not deliver a regular background. Also a grid is not completely transparent for light. Still, the combination would bring a big advantage. The larger scale of light microscopy and the usage of multiple wavelengths with fluorescence and high resolution on the same sample will make studying samples easier.

In this master thesis, a method for using cryo light microscopy and cryo elecron microscopy on the same sample is searched. To make this possible, the sample must be transferred between cryo light microscopy and cryo electron microscopy without damaging the sample. In this thesis, multiple methods to change the objective slides are tested and evaluated.

1.1 Task and requirements

In sample preparation, The Specimen such as cells are frozen inside a thin ice layer. the specimen can be stained with fluorescein before freezing for later observation with cryo light microscopy. Also a sample can be prepared to study with cryo-transmission electron microscopy (cryo-TEM). cryo-TEM allows us to see samples in an hydrated state. This is only possible in cryo-TEM, as liquid water would evaporate in vaccuum [2].

for cryo light microscopy and cryo-TEM, plunge-freezing is used in sample preparation [2] [3]. This can be done either by hand or with a plunge-freezer. Generally the same result can be produced by hand as with a plunge freezer. In practice a plunge-freezer gives more consistent results.

To successfully plunge-freeze a sample, following steps are taken: First, the slide is held by tweezers. Then a $2\,\mathrm{mL}$ water drop containing the specimen is pipetted onto the hydrophilic slide. The water droplet is blotted with filter paper, creating a thin film of water which evaporates quickly. The tweezers holding the slide is shot in cold liquid under $-140\,°\mathrm{C}$, typically liquid ethane. The rapid temperature drop freezes the water into a thin vitrificated layer of ice. vitrificated ice has no crystal structure.

A vitrificated sample is needed as ice crystals damage the specimen. Vitrificated ice is created by cooling water abruptly to temperatures below $-120\,°\mathrm{C}$ [4]. To archive rapid cooling, the slide needs a high thermal conductivity to freeze the water quickly. Also the liquid which is used to freeze the sample should not possess the Leidenfrost effect, which prevents instant contact of the sample with the cold liquid. As liquid nitrogen is possessing the Leidenfrost effect, other coolants like liquid ethane are used.

But currently, no slide exist which is suitable for plunge-freezing, cryo light microscopy and cryo-TEM. in plunge-freezing, a hydrophile surface required to archieve a thin ice layer. Additionally the thermal conductivity of the slide needs to be high for the steep temperature drop needed to create vitificed ice. In cryo-TEM, the sample needs to be extremely thin and small. A metal mesh is used to hold the sample in place. Additionally, only light elements should be used in samples as heavier electrons will disturb the results in cryo-TEM. For light microscopy, a transparent slide is not always required. Still, a smooth surface is needed. The mesh used in cryo-TEM is not suitable. The mesh is rough and has holes, which influence the reflectiveness and light which is behind the sample(???). This will heavily influence the image quality in cryo light microscopy.

To perform cryo-light microscopy and cryo-TEM in high quality, a sample transfer from one slide to another slide is proposed. The slide change must be performed at -140°C to maintain the vitrificated state of the sample. Additionally as the first slide used for plunge freezing needs to be hydrophilic, which increases ice adhesion in general. First, I investigated lipids for positive characteristics for usage as a sacrificial layer. Second, different layers are tested for low ice adhesion. Also different parameters on the mechanical setup are tested.

1.2 State-of-the-art

Ice removal is needed in multiple commercial fields. But most strategies are applicated in temperatures down to $-30\,°\mathrm{C}$. Since the ice layer in my application needs to stay vitrificated at under $-120\,°\mathrm{C}$ or lower, only few solutions are transferrable. For example the active anti-frosting strategy of heating the ice is not possible. The ice layer needs to stay intact and cannot be melted.

There are four passive anti-frosing strategies: Inhibition of ice nucleation is archieved by using surface inherent properties to prevent ice crystals from forming. Retardation of frosting removes water to prevent icing on the surface with water repellent properties such as the lotus effect. Mitigation of frost accumulation prevents already formed ice droplets from further accumulating and forming an ice layer. Last a reduction of ice adhesion on the surface prevents ice droplets to stay on the surface. Other forces like wind or gravity removes ice droplets and keeps the surface ice free [5].

Out of the four passive anti-frosting strategies, only one is applicable at cryogenic temperatures. Inhibition of ice nucleation, retardation of frosting and mitigation of accumulation only inhibit the freezing process itself. Still, the reduction of ice adhesion can be used to decrease the force needed to detach the ice layer mechanically. One material commonly used is Polydimethylsiloxane (PDMS).

PDMS is a polymer which is widely used in different applications like in fabrication of microchannels, chip manufacturing, aerospace industry and medical tools. It is used because of its properties like hydrophobility, biocompatability, electric insulating. Also PDMS is cost effective and allows rapid prototyping, molding and thin coatings. [6].

One application is the passive deicing of Aircrafts in flight. As ice can influence the air flow around the wing an the body, which induces turbulence and reduces lift. Ice protection is therefore critical for a save and stable flying. In [7], PDMS is tuned for optimal characteristics in flight. To test the surfaces, flight conditions of $0.5\,\mathrm{bar}$ and $-12\,^{\circ}\mathrm{C}$ are simulated. Fluorinated PDMS with and without silica nanoparticles are compared to aluminum, showing better resistance against ice growth. The different coatings are also examined regarding contact angle of water and surface roughness. Also the stability of the surface is relevant as ice formation and impacts can also wear down the coating itself.

2 Experiment preparation and process

(vielleicht absatz dass die Arbeit größtenteils aus praktischen Versuchen bestand???)

To find a working solution, many experiments are conducted.

2.1 Phospholipids

Phospholipids are the building block of membranes in nature. They are made of two long nonpolar carbon chains and a polar head. A membrane is a bilayer of Phospholipids with the hydrophobic carbon chains oriented inwards and the hydrophilic head pointing outwards. They are also natural detergents, as they can bind to hydrophobic waste forming an emulsion, making them removable with polar liquids like water [8].

Phospholipids are solvable in some common solvents. To apply Phospholipids, a slide is dipped into the solvent. When the solvent dries out, the lipids are binding to the surface of the slide, forming a layer with varying homogeneity. this layer can be removed with the same solvents. If the ice layer is frozen onto the lipid layer, the lipids can be solved at cryogenic temperatures, detaching the ice. But to solve this layer, a high solubility at cryogenic temperatures is required.

2.1.1 Parylene

Parylene is a hydrophobic polymer used as a coating to repel particles, including water and ice. Parylene is also biocompatible and used in medicine and biology (QUELLE?).

Parylene is not usable without a second layer on top. Parylene hydrophobicity does not allow water to spread during plunge freezing. with plasma activation, the surface is now hyrdophilic, but ice adheres to the parylene too strong to mechanically detach. also parylene cannot be solved with a solvent as a sacrificial layer.

For this reason, lipids are used in combination with parylene (Fig. 2.1). The hydrophobic chains of the lipids adhere to the parylene. The polar head allow water to spread evenly over the surface. Solving the lipids with a solvent will detach the ice layer from the slide. Parylene additionally prevents (re-) attachment through holes in the lipid layer.

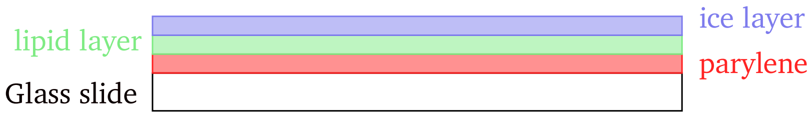


Figure 2.1: Layers of a Sample. The Lipid layer is used as a sacrificial layer. To reach the layer with a solvent, the only contact surface is to the edge. To get a fast and reliable process, a solvent with high solubility is needed.

2.1.2 Preparation of lipid coated slides

To create the slides with parylene and lipids, a cover glass ($5\,\mathrm{mm}$ diameter) is used as base. the cover glass is coated with a thin layer of parylene. The coated cover glass is dipped into lipid solution. The cover glass is dried, leaving behind a lipid layer. The prepared slides are then used in plunge freezing.

Two different kind of lipids are used: DOPC and EGG-PC. DOPC is storaged as a powder. The DOPC powder is solved in Ethanol ($25\,\mathrm{mg}/1\,\mathrm{mL}$ lipid to solvent) for application. EGG-PC is shipped solved in chloroform in two different ratios: $25\,\mathrm{mg}/1\,\mathrm{mL}$ and $10\,\mathrm{mg}/1\,\mathrm{mL}$. The solution is shipped in phioles.

The lipid solution is transferred into several small bottles. small bottles are chosen because solution forms a lubrication film on the thread of the lid. this prevents the lid from closing airtight. This leads to evaporation of the solvent over time, making the bottle unusable. In the coating process, solution often drops onto the threads, making a bottle only usable in one coating session. By splitting the solution into multiple flask, more slides can be covered from one batch of solution.

2.1.3 solubility lipids

These tests are conducted to find a fluid to solve a sacrificial layer out of lipids. (BEDINGUNGEN SACRIFICIAL LAYER? ODER KAM DAS SCHON?)

Two consecutive solubility experiments are proposed. The first experiment is conducted at room temperature. the aim is to find solvents with high solubility at room temperature. the candidates with high solubility are then tested in the next experiment at cryogenic temperature. the aim is now to find solvents with also high solubility at cryogenic temperatures. The first experiment is conducted as there are only three baths available at cryogenic temperature. therefore the throughput for experiments is limited.

at room temperature

The Solvent are chosen based on availability, freezing point and safety. The solvents are all readily available in the laboratory. Some were ordered before the test. Also all chosen solvent are save to use in a well ventilated room. The followup experiment cannot be conducted under a extractor hood as too much space is taken up with the experiment. Also the solvent or solvent mixture needs to stay liquid at around $-140\,^{\circ}\mathrm{C}$ to assure that the ice layer on top stays vitrified. The tested substances are 4-Methyl Pentene, 3-Methyl Pentene, 1-Pentene, Isopentane, 1-Propanol, Pentane and Ethanol.

Each solvent is put in a separate bottle. The lipid coated slides are prepared as previously described. For each solvent, a slide is put in the corresponding bottle. After $15\,\mathrm{min}$, the slides are removed and examined. The results are documented in a list. When all streaks caused by the lipid layer disappeared, the solvent is tested in the next experiment.

at cryogenic temperature

Solubility is temperature dependent. Most solutions are endothermic. this means energy is needed to solve another substance. Also the saturation point of the solution can change. Therefore, solubility needs to be tested at the same temperature as in application

The experiment is conducted at $-140\,°\mathrm{C}$. The solvents are given in liquid nitrogen cooled baths, which are regulated to the desired temperature. A slide is given into the cold solvent for $15\,\mathrm{min}$. Then the slide is examined for leftover streaks as before.

The freezing point of tested solvents are not all below $140\,°\mathrm{C}]$ (Table 2.1). Still, solvents with a high freezing point can be mixed with other solvents with lower freezing point to lower the freezing point of the mixture. Alternatively, the temperature can be raised over the freezing point, but this could risk the ice to loose the vitrified state.

solvent	melting point in °C
4-Methyl Pentene	-154
3-Methyl Pentene	-154
1-Pentene	-165
Isopentane	-160
1-Propanol	-126
Pentane	-129
Ethanol	-114

Table 2.1: Melting Point in °C for tested solvents.

In the end, experiments has proven that solving lipids fast and reliable is not possible with tested solvents. As all solvent lipid combinations are endothermic. finding a working solvent lipid combination is very unlikely, as almost all combination of solvent and lipids will be endothermic.

Additionally, some solvents tested are soluble in water. It is unknown whether the solvents could be solved or diffuse inside the ice layer at $-140\,°\mathrm{C}$. Therefore the ice layer could be changed in some undesired manner. if a sufficient solvent is found and the solvent is soluble in water, a potential change of the vitrified ice needs to be addressed.

2.2 Detaching ice mechanically

The next explored method is detaching the ice layer mechanically. For this, a lifting assembly is used. To make this possible, the bottom layer is engineered to reduce the adhesion of the ice. Also, as the assembly was not used in similar work before, different variables are addressed and examined.

2.2.1 Assemblies used at cryogenic temperatures

The assembly used to lift up samples is called the "finger". The finger is made of two main parts. The first part is metal rod with a slightly pointed tip (Fig. 2.2). The rod is cooled with cold nitrogen gas. Near the tip, the rod is temperature controlled with a temperature sensor and a heater. The second main part is a 3D printed part, containing the outer shell and routing of the cold gaseous nitrogen. The nitrogen is directed downwards around the metal bar in an inner mantle for cooling. then, the Gas is directed upwards flowing through an outer mantle for additional cooling. Then the gas is exits through the output.

The Gas is supplied by a liquid nitrogen tank. Heaters are placed inside the tank to evaporate the liquid nitrogen. The volume is rapidly expanding at eveporation, resulting in fast gas flow. the cold nitrogen gas is routed by $6\,\mathrm{mm}$ pneumatic tubing. at the inlet and outlet of the finger, festo connectors are fixed to allow easy maintenance. the inlet of the finger is connected to the liquid nitrogen tank. the outlet tubing exhausts the cold nitrogen into the atmosphere.

The finger is mounted on three stages. additionally the stages are mounted on a track. the three stages allow fine adjustment of the finger position in X,Y and Z axis. Also, when the finger is attached to a surface, force can be applied by moving the stages in either direction. The Finger can also be moved along the track. In use, the assembly is clamped down on the track to prevent movement. when not in use, the assembly can be moved on the track to allow easy access of the area below the "finger".

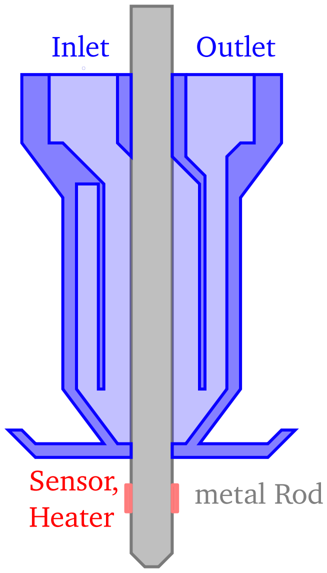


Figure 2.2: A cross-section of the "finger" assembly. The metal rod is cooled with cold nitrogen gas. the gas is routed from the inlet around the metal bar onto an outer layer to the outlet. The metal rod is temperature controlled by a temperature sensor and a heater. With HFE 7200 is applied to the tip, the finger can attach to a surface at cryogenic temperatures and apply force.

On the tip of the finger, Hydrofluorether (here HFE 7200) is applied. HFE is an oil typically used as an cryoimmersion fluid [9]QUELLE ÜBERPRÜFEN!. Besides that, it has temperature dependent abilities. At freezing temperatures, it does not freeze into a solid at once. It gets more and more viscious before it freezes completely. this temperature dependency is used to first apply the HFE at higher temperatures with low viscosity and pull on the sample at low temperatures.

In the beginning a smaller bath is used (Fig. 2.3). The Small bath contains an elevated floor as work surface. embedded in the work surface are indents which are used as container holder. those container allow transport and long term storage of samples. Three elevated baths are installed above the work station. They are used for temperature controlling and containing other Liquids or tools. Also a Haven for a shuttle system is installed. The small baths and the haven are elevated over the second floor with an insulating layer, so a temperatures over $-195.8\,°\mathrm{C}$ can be regulated. The liquid nitrogen is filled over the work surface, but not over the insulating layers to allow temperature controlling. The whole bath is insulated by Nitrogen gas flowing inside the 3D-printed shell of the bath. Then the Nitrogen gas is expelled from the brim, pointing from the outer edge radially to the rotation axis. In that way, the nitrogen gas separates the damp air in the room with the dry nitrogen gas inside the bath. Therefore ice formation inside the bath is inhibited.

This small bath and later the big bath are also used in combination with the "finger". The "finger" is positioned over the shuttle docked in the haven. The sample is fixed in the shuttle. with the stage, the position of the "finger" is manipulated. The Nitrogen gas also keeps the "finger" tip ice free.

The usage of the small bath in combination of the finger has its limitations: first, the space is small. The "finger" can be moved along the track, but the space still limits work freedom with pincers. Additionally, the smaller baths are not needed when using the finger. also the Shuttle needs to be tilted in a specific angle so docking and undocking of shuttle in the haven is possible. The work flow also allows only one shuttle at once, limiting throughput. Also Liquid nitrogen needs to be refilled often as the bath can only hold a small volume.

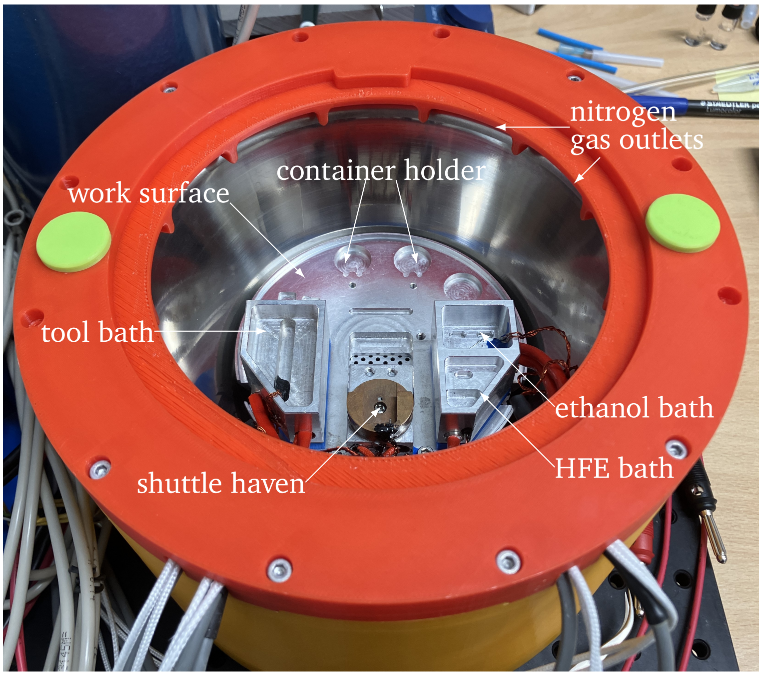


Figure 2.3: Small Bath.

During this master thesis, a second bigger bath is build (Fig. 2.4). In general, the structure is similar. It also has an elevated floor as a work surface. The work surface is fabricated out of two plates screwed together. Indents are formed by holes in the upper plate. No baths are installed, but the space is planned in for later addition. The work surface is held in place by 3D printed holders which are fixed to the brim of the metal bath. Also two harbors can be mounted for parallel work on two separate shuttles. The harbors are screwed on an aluminum block. the aluminum block is temperature regulated. between the aluminum block and the work surface, a 3D-printed insulating layer is placed. Also both harbors can be mounted either flat or in an angle, depending of the 3D printed layer. The Bath is insulated with styrofoam and a rim with holes for warm nitrogen gas is placed on top. The holes are places along the inside of the longer perimeter, so the stream covers the whole area with minimal turbulence. This also keeps the inside ice free.

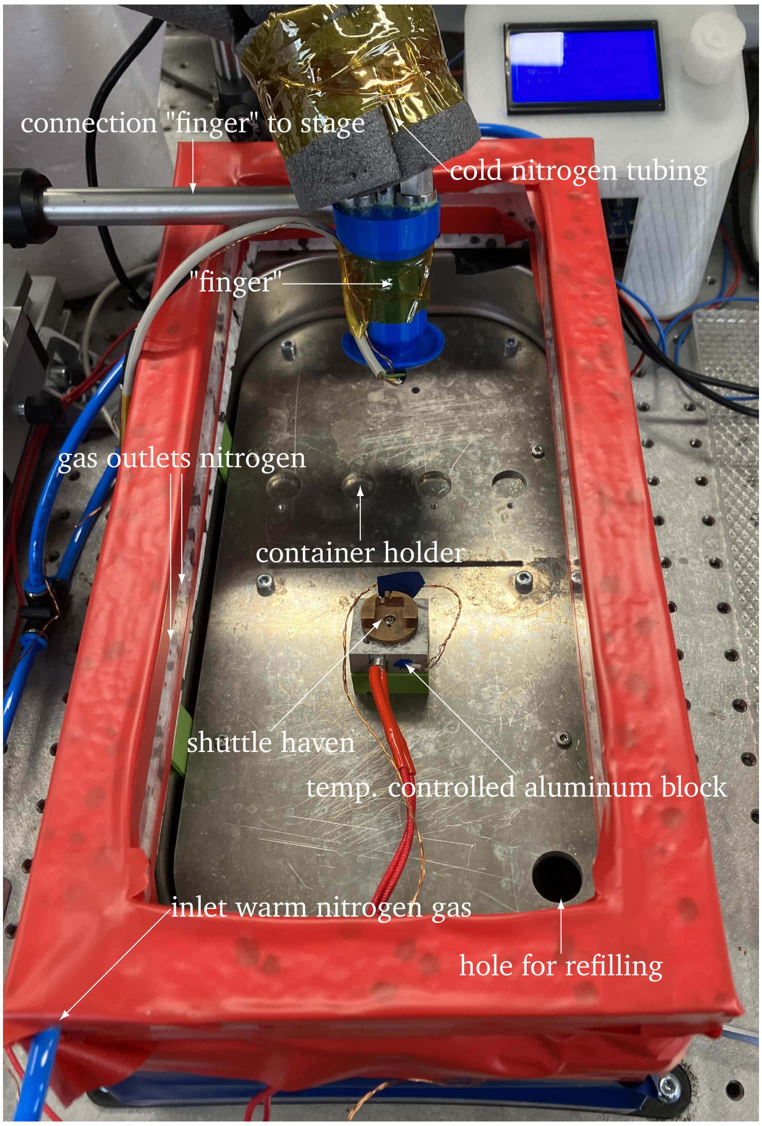


Figure 2.4: Big Bath with Finger.

To take pictures of the sample, an inverted microscope is rebuilt for cryo microscopy (Fig. 2.5). A box with a metal core is fixed to the bottom of the stage. an outer frame an the top of the box is temperature controlled. The box is supplied with cold nitrogen gas to cool the iron core. A haven is fixed to the iron core and placed over the objective. The haven is temperature controlled to $-140\,°\mathrm{C}$ to make remains of HFE liquid and distinguishable. to keep the light path between haven and objective clear from ice and fog, a component reffered to as "glasses" is used(HIER REFERENZ ZUR ZEICHNUNG VON DER BRILLE?). It contains two parallel glass slides. Warm nitrogen gas is routed beween the glass slides and the lower glass slide and objective. Additionally, a filter is used for examining fluorescent samples.

The Shuttles are also used in cryo light microscopes. The Microscope used for cryotemperatures have an additional box installed, routing Cold nitrogen gas underneath a harbor, where the sample is placed. Heaters are placed around the box and under the harbor to archieve a constant temperature. On top of the Harbor, warm Nitrogen is blown so no ice is forming inside the optical path.

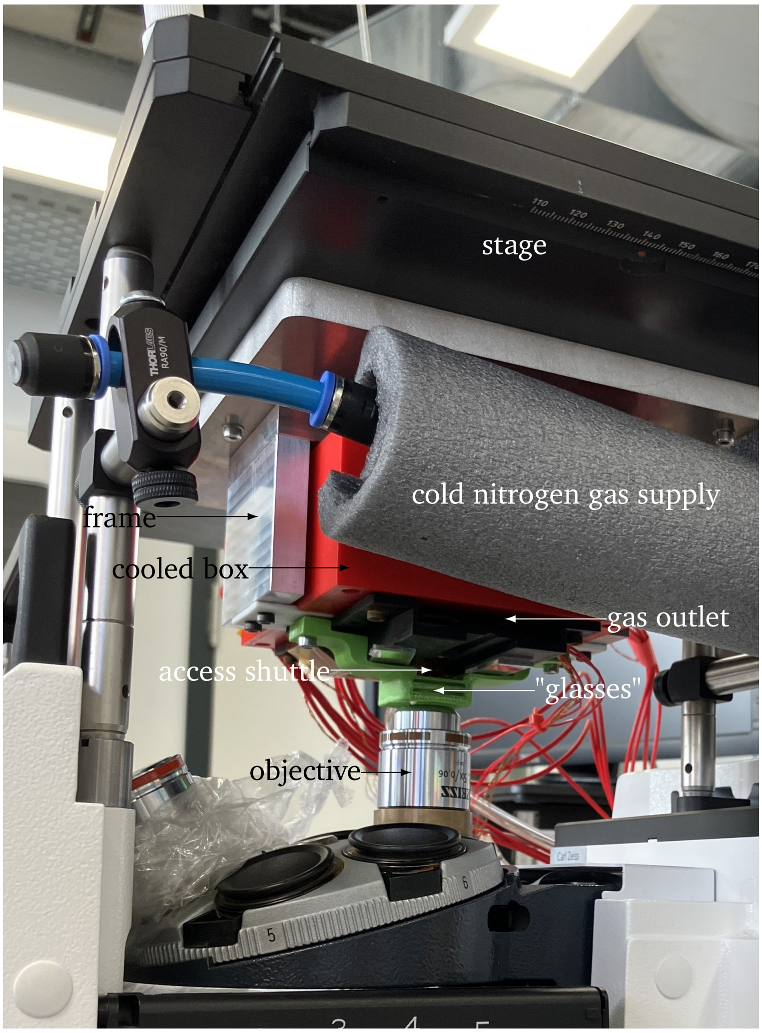


Figure 2.5: Modified microscope for cryo microscopy.

The shuttle allows easy transportation of the sample without limiting the access to the sample. The sample is clamped down by a brace, which is also reffered to as "window". The "window" is fixed with two screws to the shuttle, holding the sample in place. the "window" gives access to the top side of the sample with a center hole for microscopy and "finger". A long rod with a thread at one side and a temperature insulated knob on the other side is used to screw into the sample. This allows easy transport between bath and microscope.

On the other hand, small container with space for three $\varnothing 5\,\mathrm{mm}$ samples are used (Fig. 2.7). These are 3D Printed, modified version of other containers. a cap which is identical with the other versions of container is screwed on top with a special pencil. Inside the bath, the container fit into the indent. the cap is screwed next to the container, holding it into place. Also the container can be stored in the same container of the other version. This allows long term storage of samples with $5\,\mathrm{mm}$ diameter.

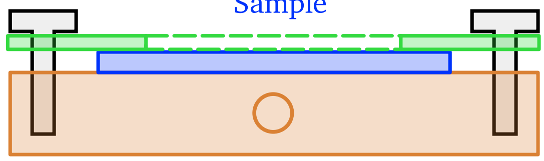


Figure 2.6: Shuttle for transporting a sample between bath and microscope. The "window" is clamped down by screws to the copper shuttle, holding the sample in place. Through the hole in the window, microscopy is done on the sample. The "finger" fit also through the "window" for pulling. For transport, a rod is screwed into the copper shuttle.

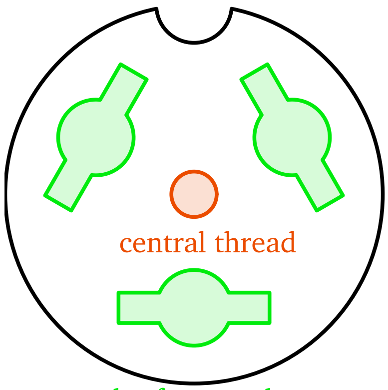


Figure 2.7: Small transport box for three samples. a cap is screwed onto the central thread. (WAS ZUM DECKEL SCHREIBEN). These boxes fit into storage units for long time storage.

2.2.2 Process

The goal is to mechanically lift a piece or the whole ice layer from the slide it is frozen to. In the whole process, the ice stays in the vitrified state. The sample is prepared in the bath. The "finger" is used to attach and pull on the ice layer with HFE. Also the microscope is used to take pictures of the sample without heating up the sample.

To try out the detachment with the "finger" in a repeatable manner, a core process is established for orientation. The sample is prepared and put in the bath. The copper shuttle part is placed in the harbor. With HFE, the sample is placed in the middle of the shuttle. The HFE helps the sample to stay in place before fixation. The window brace is placed on top and screwed down. The now prepared shuttle is transported quickly to the microscope. When the transfer is not possible within seconds, the shuttle is placed in a portable container with liquid nitrogen. After microscopy, the sample is placed into the bath unter the cooled finger.

First, if not already done, cool down the "finger" to $-140\,^{\circ}\mathrm{C}$. HFE is applied to the tip. The "finger" is lowered onto the sample while correcting the position with the stages until the HFE contacts and spreads over the sample. The temperature is reduced to $-160\,^{\circ}\mathrm{C}$ and waited until the sample and finger is cooled down. When the temperature is reached the finger is pulled up by turning the stage until detachment. Then the shuttle is transported to the microscope and the sample is analyzed.

When detachment is successful, the ice piece hanging on the "finger" is placed on another shuttle. This is done by lowering the finger onto the new shuttle and raising the Temperature to $-140\,°\mathrm{C}$.

To collect first insight, samples with parylene and lipids (as described in Section 2.1) are used first. different variables are determined which could significantly influence successful detaching. Then the different variables are examined with experiments to improve the reliability of the "finger". The different variables found in this thesis are discussed in the following sections.

2.2.3 Volume of Hydrofluorether on "finger"

Using the correct amount of HFE is important for higher repeatability. Too little HFE will not bind to the finger and sample. too much HFE results either in a thicker layer, or the HFE flows between "window" and sample. as the cohesion of the HFE is considerably lower than the sample and the finger, a thick layer is prone to breaking before the ice layer. HFE under the "window" clamp will redirect a part of the force, making the sample more stable. This makes detaching also harder.

In the beginning, the HFE was applied with a pincer.The HFE is given in a cold bath at $-140\,°\mathrm{C}$. HFE gets more viscous at colder temperatures and is evaporating much slower. The thickened HFE is now scooped with pincers onto the tip of the "finger". This method is not used later, as measuring the volume of HFE when applying is not possible.

As an effort to determine the volume of HFE a pipette is used. The HFE is pipetted at room temperature onto the cold finger. When HFE is applied onto the desired surface, around $4\,\mu\mathrm{L}$ has already evaporated. Also, only HFE on the flat surface facing the sample is usable.

Another way to determine the Volume of HFE on the "finger" is by analyzing pictures of the "finger" with HFE. The volume is calculated with the contact angle on the "finger" and the area covered in HFE. With the knowledge if the HFE spreads too much or not being attached properly to the sample, a range can be given where success is more likely. Still, other factors like temperature or the gap between sample and "finger" can influence the result.

2.2.4 Temperature

Ethoxynonafluorobutane, also called HFE 7200, is used throughout all experiments. It has a freezing point of $-138\,°\mathrm{C}$. Below the freezing point, HFE gets increasingly viscous. at a certain point, the hfe gets brittle and cracks start to form by decreasing temperature. Between the freezing point and the point of cracking, HFE is usable as glue. With a temperature near the freezing point, HFE can spread over the surface. At lower temperatures, HFE gets hard enough to hold the finger to the upper ice layer.

The temperature regulation of the finger has three modes: First the "unglue" mode which regulates the Temperature to $-140\,°\mathrm{C}$. HFE has a low viscosity, which allows the application of HFE. Also detachment without transferring force is possible. Second, in "glue" mode the shuttle and the finger are cooled to $-160\,°\mathrm{C}$. HFE hardens and force can be applied to detach the ice layer. Third, "thaw" cleans the finger by heating the tip to $20\,°\mathrm{C}$, evaporating everything stuck on the "finger".

As HFE is getting harder with temperature, lower temperatures in the "glue" state could allow higher forces on the ice layer. Still, the point where HFE starts to crack will decrease the tensile strength. To test this hypothesis, HFE is examined at temperatures until $-170\,°\mathrm{C}$.

2.2.5 Direction of force

The direction of force can increase the likelyhood of detachment. Tensile mode and shear mode can be applied by the finger. Tensile mode is the easiest to apply with the finger. Also HFE is able to withstand some tensile forces. But for separating layers, this mode could take more force than applying tensile forces, depending on the bottom layer. Still, HFE stability to shear stress is not known. Additionally, the sample is clamped down to the top. This does not allow the ice surface to slide off without breaking.

In some experiments, the shuttle is tilted around 15 degrees for easier access to the shuttle. The finger is also tilted so the tip surface is parallel to the sample. To apply force, the finger is pulled by stages in either X, Y, or Z direction. For each direction, the stress is split into tensile and shear stress. In Z direction, mostly tensile stress is applied (Fig. 2.8 (a)). in X direction, mostly shear stress is applied (Fig. 2.8 (b)). In Y direction, only shear stress is applied, but will result in shattering of the ice layer, probably taking additional force. For this reason, only X and Z direction are tested.

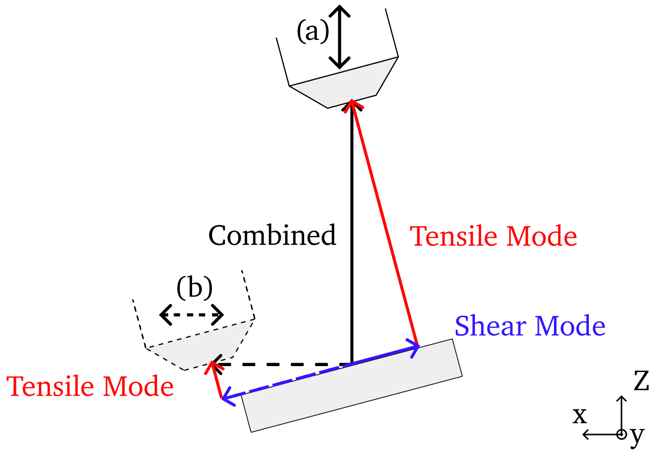


Figure 2.8: Tensile vs Shear mode

2.2.6 ice structure

DER TEIL BRAUCHT NOCH ARBEIT!!!!!!!

To lift off a piece of the ice layer, the ice layer must be broken in some way. thicker ice layers are expected to be harder to break than thinner ones due to the bigger cross section. Also amorphic vitrified ice is expected to be more stable than crystallized ice.

Initially, to save time for experiments, the samples are freezed by Hand in liquid nitrogen, as described before. However, the ice layers are less consistent compared to plunge freezing, resulting in mostly thicker ice layers compared to plunge freezing. also as the sample is frozen in liquid nitrogen, the leidenfrost effect is inhibiting the formation of vitrified ice.

In experiments, the used glass slide and freezing method does not produce vitrified ice. Therefore the influence of vitrification is not observed. The thickness of the ice is also not measured directly.

To compare the influence of hand freezing and plunge freezing, results of lifting off samples frozen with both methods are compared. No other factors are varied in those experiments. In the end, hand freezing and plunge freezing did not make a difference. Therefore, hand freezing was also applied in future experiments, as this effect is determined as neglegtable compared to other factors.

SAMPLE INTEGRITY

2.2.7 positioning

Before attaching the "finger" to the sample, the "finger" is positioned with the stages. At positioning, two different errors can occur: first, the finger is incorrectly positioned over the sample. the HFE is partially on the "window" brace. The second error is a too small or too big gap between "finger" and sample.

An incorrectly positioned finger is expected to be a major error. Depending on the overlap, the force is transferred mostly to the brace than to the sample. This will lead to an unsucessful detachment. With the setup of three stages, the positioning is easily corrected. Alternatively, a "window" brace with a bigger central hole makes positioning easier.

The gap between sample and "finger" is sometimes hard to estimate. The HFE volume can influence the preferred gap size to avoid pressing the HFE between sample and "window". On the other hand, HFE is shrinking between temperatures used at attaching and pulling.

(HIER WEITERSCHREIBEN ODER BEI ERGEBNISSE??)

2.3 PDMS

PDMS is a polymer used in coatings for passive deicing. It is hydrophobic and has a low surface energy. Also it can be coat spinned into a thin layer to form the thin coat. Also it is widely available and tunable. To use PDMS in plunge freezing, the PDMS is plasma activated. The requires a Plasma generator.

To create different PDMS mixtures, Dowsil Sylgard 184 Silicone elastomer is used[10]. The PDMS kit has two components. The base coat component is highly viscious, whereas the curing agent is liquid. The Specified mixture ratio is 10 base coat to 1 curing agent in weight (10:1). In some applications, other mixture ratios are used and additives are added for tuning PDMS. In my research, I focussed on tuning the mixture ratio of base coat to curing agent.

PDMS properties are also temperature dependent. In [11], multiple characteristics are determined for cryogenic temperatures. The PDMS is prepared with the standard mixture ratio of 10:1. The compressive strength increases with lower temperatures until $-123.15\,^{\circ}\mathrm{C}$. At this temperature, the compressive strength reaches a maximum of $224.50\,\mathrm{MPa}$ in average. At lower temperatures, the PDMS gets brittle. At $-150.15\,^{\circ}\mathrm{C}$ PDMS has a compressive strength of $106.99\,\mathrm{MPa}$.

The "finger" requires a temperature of $-160\,^{\circ}\mathrm{C}$. Therefore, the temperature drops enough to make PDMS of 10:1 mixture ratio brittle. Generally, a brittle PDMS surface helps to detach the ice layer. Tensile forces loosens the PDMS under the Ice. The PDMS layer breaks within itself, loosening the ice layer from the rest of the sample. Still, an extreme brittleness is needed, which may not be reached. With PDMS of other mixture ratios, the brittleness could change, including the temperature dependency.

2.3.1 Plasma surface treatment of PDMS

Plasma curing is commonly used as PDMS treatment. For example, it is used to bond two PDMS surfaces together [12]. It is also used for increasing wettability and adhesion. Plasma treatment is changing the chemistry of the polymer chains on the surface. Charged Oxygen Ions are deposited on the surface. these Ions make the surface temporarily hydrophile and increasing water adhesion. The Ions change the structure of the PDMS is permanently by oxidation. In some cases, cracks form as the surface oxidizes to a silica like form.

In [13], the influence of different gasses on PDMS plasma treatment is examined. Oxygen, Nitrogen, Argon and Helium are compared on the effect on wettability, adhesion and cracking. Thin PDMS sheets are used with unknown composition. They found similar results between gasses. All gasses produced a thin and brittle surface with cracks and high wettability. Based on these results, used gas is not a significant factor for plasma activation. Therefore using only air (mainly a mixture of nitrogen and oxygen) is sufficient to determine the effect of plasma treatment.

In [14], the influence of plasma treatment on Mixture ratios of 50:1 to 100:1 is described. A thin layer of those PDMS mixtures is put onto a preformed PDMS piece with lower mixture ratio. The preformed piece is used to apply shear stress to the surface by stretching the lower PDMS form piece. Therefore only tensile force are examined. It shows no significant difference between described mixture ratios. It also shows that higher plasma treatment leads to more brittle surfaces, reducing the force needed to break the PDMS Layer by $90\,\%$.

Also known are the adhesion forces on PDMS from 1:3 to 50:1 mixture ratio. In [15], PDMS is mixed in different weight ratios. The mixture is given into a mold and afterwards fully cured. An ice block is frozen on top of the PDMS. Using a pulling machine the maximum tensile and shear forces for detaching ice from PDMS are determined. Results show that mixture ratios 10:1 to 1:3 have significant lower adhesion forces on ice. At for example at 2:1, the shear mode is just under $20\,\mathrm{kPa}$ and the tensile mode around $30\,\mathrm{kPa}$.

Still, the effect of plasma activation under 50:1 is unknown. The plasma activation has two effects: first, it can increase the adhesion on ice to PDMS. second, the PDMS changes its structure, which could increase as well as decrease the strength of the PDMS layer. In the following, the effect of plasma activation on PDMS are examined. This is done at room temperature to speed up the process.

2.3.2 Preparation of PDMS samples

All PDMS samples are prepared in a similar way. The preparation starts with weighting out the desired amount of base coat and curing agent. The mixture is stirred intensively. The mixture is placed under a vacuum bell to gas out all air bubbles from stirring. Meanwhile the glass covers are cleaned with ethanol or isopropanol and dried. Afterwards, the PDMS mixture is coat-spinned onto the glass covers. Then the coated cover glasses are baked in the oven.

For 1:2 base coat to curing agent weight ratio, the PDMS mixture is lightly viscious. A vacuum of $30\,\mathrm{min}$ is drawn for degassing. A coat spinning time of $120\,\mathrm{min}$ at $3000\,\mathrm{RPM}$ results in a smooth surface on all used slides. The baking time of at least $24\,\mathrm{h}$ by $80\,^{\circ}\mathrm{C}$ is needed. For shorter baking times, plasma treatment has a slighlty different effect. Normally, touching a treated area will neutralize the effect of plasma treatment like hydrophility only locally on the touched surface. But here, touching the surface leads to the complete neutralization of plasma treatment when touching. In this work, the effect is undesired.

For 4:1 base coat to curing agent weight ratio, the PDMS mixture is more viscious. The Vacuum and coat spinning are the same for $30\,\mathrm{min}$ vacuum and $3000\,\mathrm{RPM}$ for $120\,\mathrm{min}$. But a baking time of $30\,\mathrm{min}$ at $80\,^{\circ}\mathrm{C}$ is already sufficient to harden the PDMS.

For 50:1 base coat to curing agent weight ratio, the PDMS mixture is as viscious as the base coat. A longer vacuum of $1\,\mathrm{h}$ is needed to air out all bubbles. The coat spinning speed and time is increased to $3500\,\mathrm{RPM}$ for $180\,\mathrm{min}$ (NACHSCHAUEN). Also a longer baking time of $20\,\mathrm{h}$ at $80\,^{\circ}\mathrm{C}$ is needed to harden the PDMS.

In the coat spinning setup, each slide is coat spinned in succession of each other. Each coat spinning process needs 2 to 3 minutes. This results in a time consuming process. To speed up the process, rectangular cover glass with 20x20 and 24x40 are coat spinned. afterwards the cover glass is split in multiple smaller pieces.

The process is the same described as before for each PDMS mixture ratio, but with some adjustments. Before coat spinning, the glass is scratched with a diamond pencil. The scratches in the glass are weak points for breaking out smaller parts. Tape is put over the scratched glass. The glass is put into the coat spinner with the taped side facing down. The PDMS is coat spinned onto the exposed glass side. The baking process is as previously described. After baking, the glass is broken into smaller pieces. This is done by dragging the attached tape over a table edge. Then the glass is fixedto the table with PDMS facing up. The PDMS is covered with (????) to keep the PDMS clean. As the sticky side of the tape is not facing the table, the tape ends are additionally taped to the Table. Then smaller sample pieces can be broken off, The pieces are loosened from the tape by forcing a flat pincer between glass and the tape it is attached to.

This method has advantages as well as drawbacks to coat spinning small glass pieces separately. First, time is won by coat spinning, as one big glass can be split into several smaller ones. The Sample can stay fixed at the table without risking damaged. The samples can be broken off shortly before the experiment. But with hand scratching, only irregular and rectangular shapes can be won out of the bigger glass piece. also there is a significant loss of samples. This is by one part by breaking the sample. Some cracks do not follow the scratch, which leads to too small samples. Also in the process of loosening a piece cracks can form, making the piece too small for use.

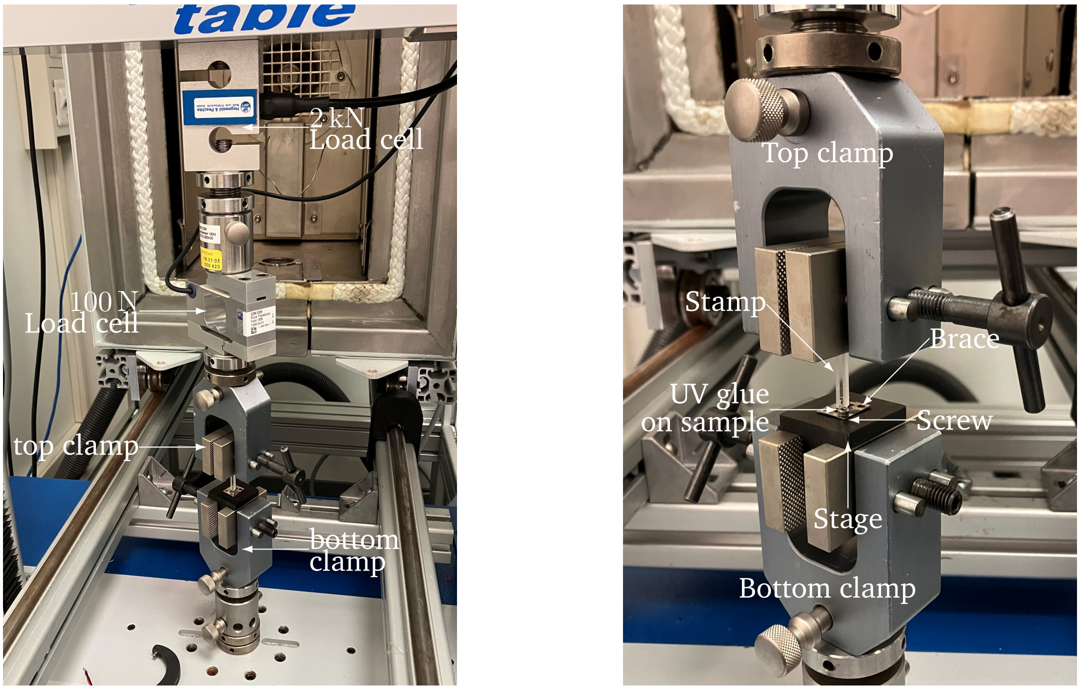
In the end, these two different fabrication methods are used for different experiments. The small round cover glasses are used in combination with the finger. The regular pieces are easier set up with the shuttle. The glass pieces broken out of a big cover glass are used under the pulling machine at room temperature. In this setup, fluctuations in size of the glass pieces are easier to handle and the clamp has more flexibility.

Setup of the pulling machine

To test the tensile strength of different PDMS mixtures and the effect of plasmacuring, a Pulling machine (NAME RAUSFINDEN)is used. On the top part, two load cells are installed (Fig. 2.9a) The upper load cell is rated for $2\,\mathrm{kN}$(ÜBERPRÜFEN). the lower load cell is rated for up to $100\,\mathrm{N}$. As the upper one is extremely stiff and the forces are $\ll 100\,\mathrm{N}$, the upper sensor is assumed as inflexible. On both the upper and lower part, two clamps are fixed onto the machine. On the bottom clamp a 3D-Printed stage is used for fixing on the sample.

On the bottom, a 3D-Printed stage is clamped (Fig. 2.9b). The stage has four holes with threads for screws . Between the threads, the sample is placed. A brace similar to the "window" is fixed with screws onto the sample. A rectangle shape hole gives access for the UV-glue and the stamp.

The Process of a pull test starts with clamping a new stamp on the top. Then the sample is plasma treated. After treatment, the sample is quickly transported to the pulling machine and fixed to the stage with screws. The stamp is aligned to the sample. When alignment is finished, the UV glue is given onto the stamp. the top



(a) (b)

Figure 2.9: Setup for tensile tests of a thin PDMS layer. The Sample is placed on the stage. A brace is screwed over the sample to hold it in place. A stamp is fixed to the top clamp. The UV Glue is applied between stamp and sample and cured with 3 minutes UV Exposure. With the $100\,\mathrm{N}$ load cell, the force is measured over distance pulled. The load on the $2\,\mathrm{kN}$ load cell is neglectable as the forces measured are $\ll 100\,\mathrm{N}$.

is lowered on the sample. The UV glue is cured with UV radiation for $3\,\mathrm{min}$. After gluing, $3\,\mathrm{min}$ are waited until forces settle resulting from curing and heat. Then, the pulling machine pulls with constant speed and measures the stress strain curves. The process is ended manually. The now separated stamp and sample are stored for analyzation under a microscope. To calculate the maximum tensile stress, the area of the glue on the sample which is still attached to the stamp is measured.

2.3.3 detaching ice from PDMS

Now, different PDMS mixture ratio with different plasma activations are tested at cryogenic temperatures. With previous setup, plasma activation of 1:2 mixture ratio PDMS resulted into a more stable layer with more adhesion. Also 50:1 is tested with high plasma activation. The tensile stress of 50:1 mixture ratio is $60\,\mathrm{kPa}$ [15]. [14] suggests that strong plasma activation over $3\,\mathrm{min}$ results of a decrease of adhesion strength of around$90\,\%$. Additionally, 4:1 mixture ratio with high plasma activation is tested.

Two different setups are used. First, the setup with "finger" and "bath" described in section 2.2.2 is used. Second, for additional data, a modified version of the setup with the pulling machine is used. This allows the measurement of the actual tensile strength on the sample.

Setup pulling machine for cryogenic tests

To allow tests at cryogenic temperatures with the pulling machine, the setup is modified to allow fitting a bath and the "finger" to the pulling machine. The big bath is used with a flat harbor. An outer frame is used and modified to hold the bath. The bottom clamp is removed and the Bath is fixed over the bottom attachment. The top part is used with the same load cells, but with a bigger clamp. The "finger" 3D printed shell modified with a flat outer profile for clamping and clamped onto the top. $90°$ festo tubing is used to connect the cold nitrogen gas supply. A power source and a temperature regulator is again used.

BILD

Compared to the "finger" setup with stages, the finger only moves up and down. The Alignment is done by moving the bath by loosening the screws on the frame. Also new "window" parts are waterjet cut. The new "window" has a bigger central hole for easier alignment.

As the pulling mashine is set up in a separate room, the samples are prepared in the laboratory next to the microscope. The small bath is used for sample preparation. The process is the same as all detachment trials with the finger.

3 Findings

3.1 Lipids

In the previous chapter, the method of using a sacrificial layer to detach ice is discussed. For this, lipids need to be solved at cryogenic temperatures. As not every lipid is solvable in all solvents, an experiment is conducted to obtain solvents at room temperature. then the best solvents are tested at cryogenic temperatures.

The solubility of lipids at room temperature in different solvents are determined. For this experiment the cover glasses are coated with lipids. Then a first reference image was taken. Then the cover glass is given into a small container with the potential solvent. After 15 minutes, the cover glass is removed and compared under the microscope with the reference picture. If streaks created from lipids are still as visible as before, the lipids are categorized as insoluble in this solvent. If the streaks partially dissapeared and/or are less visible, the lipids are categorized as partially soluble in this solvent. Last if the streaks completely disappear, the lipids are assinged as soluble in the solvent (Table 3.1).

Figure 3.1: Example of a $\varnothing 5\,\mathrm{mm}$ cover glass with lipid residuals on the surface.

This experiment shows that each three different solvent exist for EGG-PC and DOPC with high solubility (Table 3.1). Following these results, solvents categorized with "soluble" are tested regarding solubility at temperatures of $-140\,°\mathrm{C}$. As not all solutions are liquid at $-140\,°\mathrm{C}$ (Table 2.1), they are tested at higher temperatures above their melting point, as mentioned in chapter 2.1.3. In addition they are tested as mixtures with other solvents with a lower melting point. Additionally liquid ethane is tested as solvent. Ethane was not tested at room temperature, as the boiling point is at $-88.6\,°\mathrm{C}$ [16].

potential solvent	solubility EGG-PC	solubility DOPC
4-Methyl Pentene	soluble	N/A
3-Methyl Pentene	slightly soluble	insoluble
1-Pentene	insoluble	insoluble
Isopentane	soluble	slightly soluble
1-Propanol	soluble	soluble
Pentane	soluble	insoluble
Ethanol	N/A	soluble

Table 3.1: Result of solubility tests at room temperature. Soluble indicates solvents which are able to visibly solve all lipids off a cover glass. slightly soluble indicates solutions which are able to solve lipids with residuals. Insoluble indicates no visible removal of tested lipid.

In the experiment, no tested solvent was able to completely solve lipids at $-140\,^{\circ}\mathrm{C}$ and within $15\,\mathrm{min}$ (Table 3.2). Also streaks of applied lipids did not only stay partially behind, but also new streaks appear on the glass slides. This means that some lipids redistributed on the same glass slide.

Using solvents to remove a sacrificial layer, a high solubility is required. In practice, the sacrificial layer is completely covered by the ice layer except the edges. Therefore area of contact with the solvent is small, slowing the process considerably. Additionally, as the ice layer needs to stay vitrified. The temperature cannot be raised over $-140\,^{\circ}\mathrm{C}$ to speed up the process.

The solving process of lipids proves to be endothermic. This means that heat is needed to solve lipids, so cold temperature heavily decrease solubility QUELLE DENNIS ODER SO. This effect was observed over the last experiments by all solvents to varying degree. It can be assumed that the majority of solvent lipids mixtures are endothermic which is very disadvantageous for finding a potential solvent lipid candidate. Strongly exothermic solvents could heat up the ice enough to crystallize the ice. So weakly exothermic solvents would be optimal for this task.

Solvent	Result
Pentane	soluble at $-125\,^{\circ}\mathrm{C}$
4-methyl pentene	insoluble
1:1 volume ratio HFE to 1-Propanol	did not mix, slightly soluble
Liquid ethane	insoluble

(a) EGG-PC

Solvent	Result
1:4 volume ratio 1:2 molar ratio Ethanol to Isopentane	slightly soluble
1:2 volume ratio 1:1 molar ratio 1-Propanol to Isopentane	insoluble
Isopentane	slightly soluble
1-Propanol	at $-130\,^{\circ}\mathrm{C}$ slightly soluble
Liquid ethane	insoluble

(b) DOPC

Table 3.2: in 3.2a for EGG-PC, no sufficient solubility at -140°C was found. In 3.2a, DOPC was tested but also no proper solution was found.

3.2 Detaching ice mechanically

TODO

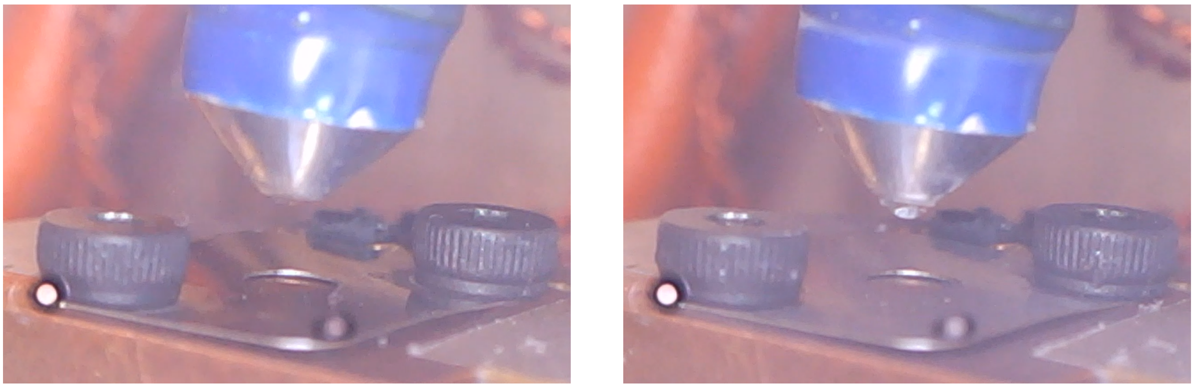
For this section, cover glass coated in Parylene are used as object slide. The slide is then dipped in solution containing lipids for a lipid coating. A ice layer with fluoriscine is frozen with either plunge-freezing or using a pincer and liquid nitrogen. Additionally, the "finger" is used as tool to try lifting off a piece of ice from the frozen layer on top of the lipids. In the next sections, different variables are examined and tested.

3.2.1 Volume of Hydrofluorether

First the volume of HFE is evaluated. High dosages of liquid HFE can spread underneath the frame holding the sample, leading to an inefficient force distribution. Also a thick glue layer take less tensile force, leading to a reduction of maximum force on the sample. Too little HFE will not connect the finger to the sample. Additionally, the dosaging of glue ia found to be a challenge.

The HFE is dosaged with a pipette onto the tip of the "finger". While pipetting, around $4\,\mu\mathrm{L}$ of HFE is evaporating. Based on this knowledge, dosaging $4.10\,\mu l$, $4.30\,\mu l$ and $4.50\,\mu l$ is compared and a video is taken for later comparison.

The videos show that pipetting HFE is not reliable. The visible HFE volume on the tip does not correlate with the dosaged volume. On reason is a difference in the volume of HFE evaporating while applying. Also some HFE may be placed on the side of the tip. When using the finger, only the HFE on the flat tip is effective.



(a) lower limit HFE volume

(b) upper limit HFE volume

Figure 3.2: Visual representation of the range of dosages which are the most reliable. less HFE than the lower limit is more likely to not attach to the sample properly. more HFE than the upper limit will spread under the "window" brace or results in a thick HFE layer with less tensile strength.

Still, a visual estimate for the correct glue dosage is made by calculating the drop volume out of camera images. Two exemplary Pictures of an upper and lower limit of glue dosages is picked (Fig. 3.2). Then the volume is calculated with a formula for the volume of a spherical section. All needed components are calculated out of the estimated contact angle of the glue $\alpha \approx 45$ and the tip diameter of $d = 1.68\,mm$. For the lower range a reduction of d by a factor of $\frac{2}{3}$ is assumed as the drop is not covering the whole tip. The resulting volume range of the HFE is $0.11\,\mu l \gtrapprox V \gtrapprox 0.38\,\mu l$.

3.2.2 Temperature over applied force

The temperature dependent viscosity of HFE is used to attach and detach from the surface of the sample. With decreasing temperature, the viscosity of HFE increases. At some point, HFE forms cracks and gets brittle. To optimize the applied force at detaching, a temperature is needed which gives HFE high viscosity but does not form cracks at the same time. The determined temperature is then set for the "glue" state described before.

The viscosity and crack formation of HFE are tested. for this, HFE is given into a small temperature controlled bath. the temperatures -150°C, -155°C, -160°C, -165°C and -170°C are compared. Also changes in HFE when cooling and heating up are observed. A needle is put in the HFE to induce forces and subjectively test the viscosity of HFE at all temperatures.

At $-150\,°C$ to $-155\,°C$, the HFE is still only lightly viscous . the needle is not held up by the HFEs viscosity. At $-160\,°C$ to $-165\,°C$ the HFE is viscous enough so that the Needle is hold up by the HFE. The Needle can be pulled out and the HFE is closing the gap. Also with enough force, the Needle can penetrate the HFE. also no cracks formed so far. At $-170\,°C$, HFE hardens further. The HFE is still viscous. Wiggling the Needle in the HFE can result into cracks. Then the Needle can be easily pulled out. Under $-170\,°C$, Cracks form without inducing forces in the HFE.

Heating the cracked HFE up leads to the cracks eventually disappearing. at $-165\,°C$, first cracks disappear, but a many remain. The cracks left are still lowering the mechanical stability of the HFE. Heating up to

$-160\,°C$ results in less cracks, but some still remain. A temperature of $-150\,°C$ will result in cracks completely disappearing.

At $-165\,°C$, maximum load can be applied. Higher temperatures result in lower viscosity, which lowers the maximum stress before HFE breaks. At $-170\,°C$, cracks will form, lowering the maximum stress.

To test how applicable the temperatures are with the "finger" setup, pulling tests are done as described in section 3.2 except the temperatures of the finger is lowered to $-165\,°C$ and $-170\,°C$ in the "glue" state. With attention to proper insulation and no leakages of cold nitrogen gas, $-165\,°C$ is quickly reached and is held stable by temperature regulation over a time span of minutes. Still, the setup is not very reliable as new leaks can form and changing of tanks/tubings can lead to new leaks. These leaks are spotted only when the finger is already cooled. To fix leaks, the setup needs to warm up. $-170\,°C$ can sometimes be reached with the finger, but holding the temperature stable is not possible.

When cooling down to the desired temperature with the "glue" state, liquid nitrogen is refilled to increase cooling. When refilling and cooling at the same time, rapid cooling happens when liquid nitrogen touches the shuttle directly at refilling. With this, the Temperature can shortly drop below $-170\,°C$. This induces cracks in HFE, lowering the tensile strength. If this happens, the Sample and finger can be heaten up to the "unglue" state at $-140\,°C$ and the cracks disappear. Then cooling can start again.

In conclusion, the finger "glue" temperature is most effective at $-165\,°C$. Still, the reliablility suffers trough leaks, longer tubing and inproper insulation. For better repetition, a temperature of $-160\,°C$ is also used in experiments.

3.2.3 Tensile mode vs Shear mode

THIS NEEDS MORE LOVE

As mentioned before, Force can be applied by moving the stage in either X, Y or Z axis. In the tilted position of the harbor, moving in Z axis splits in mostly tensile mode but also shear mode.

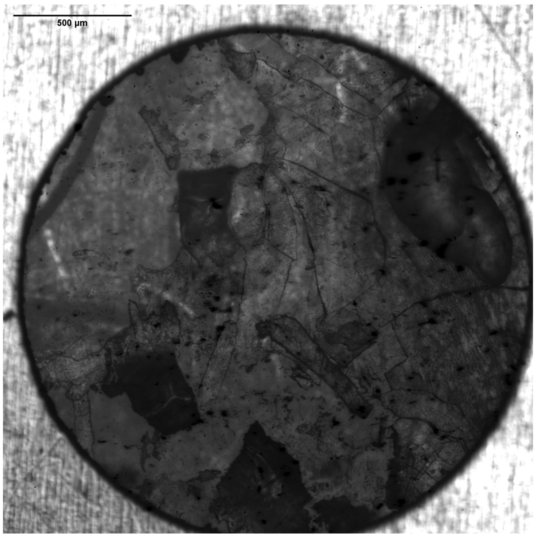
Pulling tests are compared along the X and the Z axis. Generally, way less force is transferred when moving along the X axis. this is feelable with the resistance of the stage and hearable when the HFE detaches.

3.2.4 Detaching ice structure

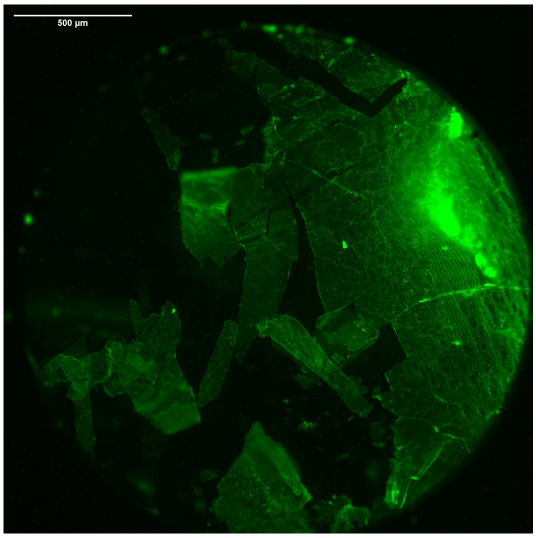
Different ice structures and thickness results in different stability of the ice layer. The freezing process has an influence on the formation of the ice structure. TO compare plunge freezing by a plunge-freezer to the process by hand, samples with lipid coated slides which are prepared with a plunge freezer are compared to samples frozen by hand.

Next observed possible factor is the thickness of the ice layer. In the following, samples freezed with a plunge-freezer are compared to samples freezed with a pincer in liquid nitrogen.

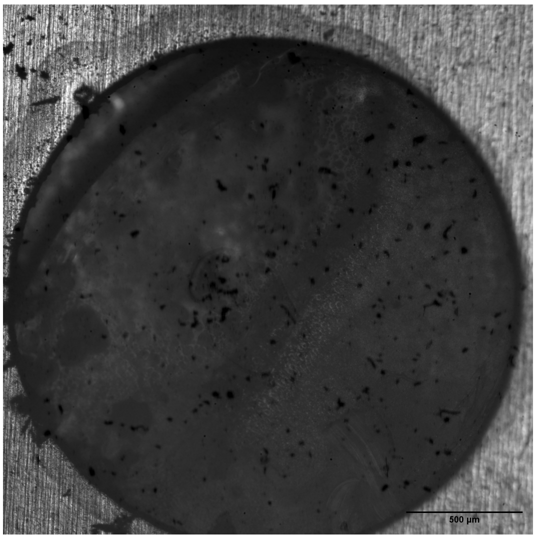
The results are categorized in 4 categories: Not successful pulls don't have visible changes of the flourescent ice layer, Partially successes are visible breaks or clear movement of ice parts on the ice layer, Successful liftoff is a missing piece and a visible piece on the finger, which could be used for future steps. In the results, there is no difference between Hand freezed and plunge-freezed samples regarding detachability. Therefore Ice thickness is not a factor which makes detaching ice easier. As both methods don't show success in detaching



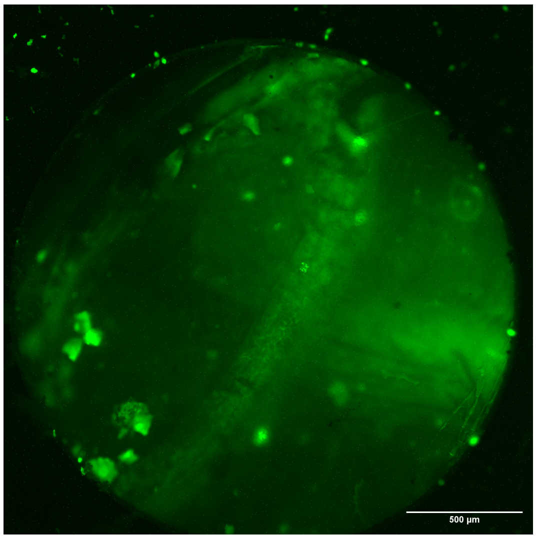
(a) Sample with a broken ice layer in true light



(b) Sample with a broken ice layer with fluorescence filter



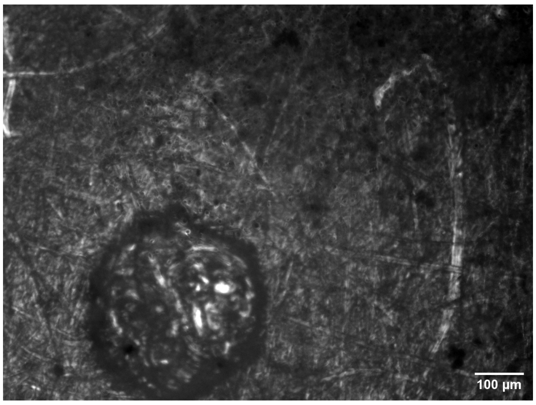
(c) Sample with continuous ice layer in true light



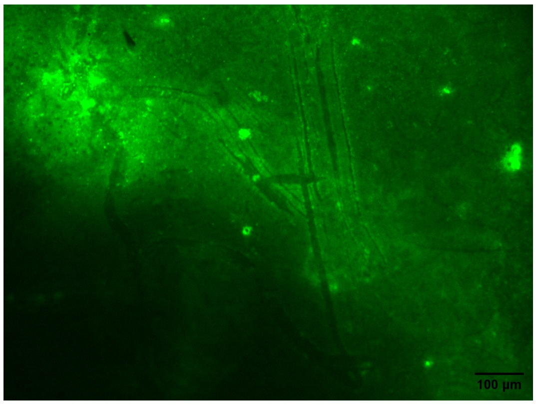
(d) Sample with continuous ice layer with fluorescence filter

Figure 3.3: Two different examples for hand freezed ice layers on samples.

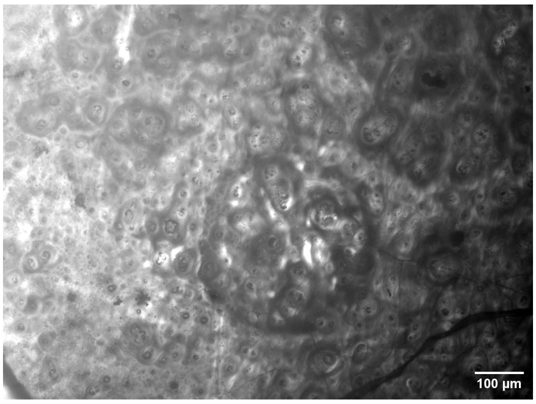
ice pieces, it could still be a relevant factor but not a thing which should make a certain solution magically work xD



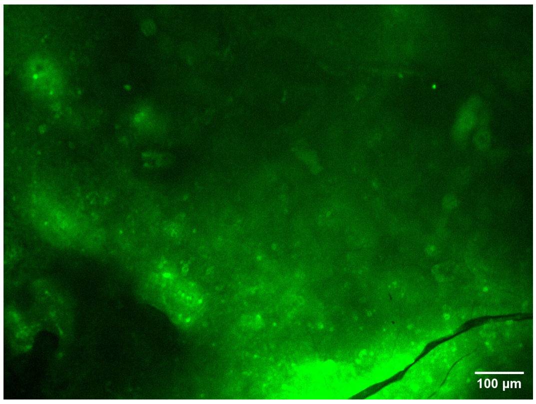
(a) Sample with thin continuous ice layer in true light



(b) Sample with thin continuous ice layer with fluorescence filter



(c) Sample with thick continuous ice layer in true light with air bubbles



(d) Sample with thick continuous ice layer with fluorescence filter with air bubbles

Figure 3.4: Two different examples for hand freezed ice layers on samples.

Category	Hand-freezed	Plunge-freezed
count executed tries	4	4
unsuccessful	3	3
breaks/movement of ice	1	1
piece lifted with finger	0	0

Table 3.3: Comparison of detachability between hand-freezed and plunge-freezed samples

3.2.5 other observed error sources??

During experiments, other error sources are found.

Zu viel abstand zu oberfläche

Integrität oberfläche

Finger kühlt zu langsam ab.

Wrong positioning, forming of ice

The formation of Ice inside the bath is largely inhibited through the Nitrogen Gas inlets. The nitrogen is forming a barrier to the atmosphere which contains humidity that turns into ice at cold temperatures. However, through turbulence and the finger, some ice can form inside the bath.

In some experiments, high buildup of additional ice is observed on the sample. The additional ice is looser, reducing the possible grip onto the desired ice layer. The formation is traced back to a leakage in the finger. The tolerances between 3D printed part and metal bar should be able to seal the gap airtight. But trough changing out parts, the tolerances are looser, allowing a weak cold nitrogen current right on the sample. There are two possibility where the humidity itself is coming from: The cold nitrogen gas itself could contain some humidity. Second through additional turbulence, more air is sucked through the barrier. The air is directed directly on the sample, causing more ice buildup. To avoid this, the gap is sealed tight with tooth silicone????

3.3 PDMS

To speed up the process of finding the right balance of PDMS Mixture ratio and plasma curing, experiments at room temperature done. For this, a pulling machine is used. First the sample is prepared. Then, the sample is clamped to the pulling machine. Then a plexiglass stamp is aligned on top of the sample. With UV glue, the stamp is glued to the PDMS layer on the sample. Before gluing, the Force and distance is set to zero on the pulling machine. After gluing, a couple minutes are waited so no further stress changes are ongoing from the gluing process. then the Machine is pulling on the sample with constant velocity. After detachment, the measurement is stopped. Afterwards, the stamp and the layer are analyzed under a microscope. The area is determined. With the maximum force and area, the maximum stress is calculated. Each experiment is repeated multiple times.

3.3.1 experiments at room temperature

In between experiments, small variations are made: two different stamps are used, one has an area of $2\,\mathrm{mm}$ x $3\,\mathrm{mm}$ and another stamp is $3\,\mathrm{mm}$ x $3\,\mathrm{mm}$. Since the area is measured afterwards, this should not have a significant effect on the results. Also, in the beginning, while waiting of the stress changes to subside, the pulling machine was inactive. with this, the Force before pulling will be higher as zero. Before pulling, the machine is set back to zero. After pulling, there is an offset between the neutral value and the value before because of zeroing, so the offset needs to be corrected. To avoid this, the pulling machine is set to zero force while waiting instead. Then no offset correction is needed. The offset correction and new method increased the accuracy between pull tests.

To verify the setup, samples coated with 4:1 and 1:2 curing agent to base coat weight ratio and uncoated coverglass used as slides are compared. The results show 2:1 mixture ratio with $87.3 \pm 19.9\,\mathrm{kPa}$ is easier to detach than 1:4 mixture ratio with $429.1 \pm 5.1\,\mathrm{kPa}$ (Fig. 3.5). Also glass without PDMS takes up a lot more tensile stress with $1161.5 \pm 111.5\,\mathrm{kPa}$. sometimes the machine is able to break the glass. Under the microscope, it is not visible if the PDMS layer itself was lifted from the glass or not.

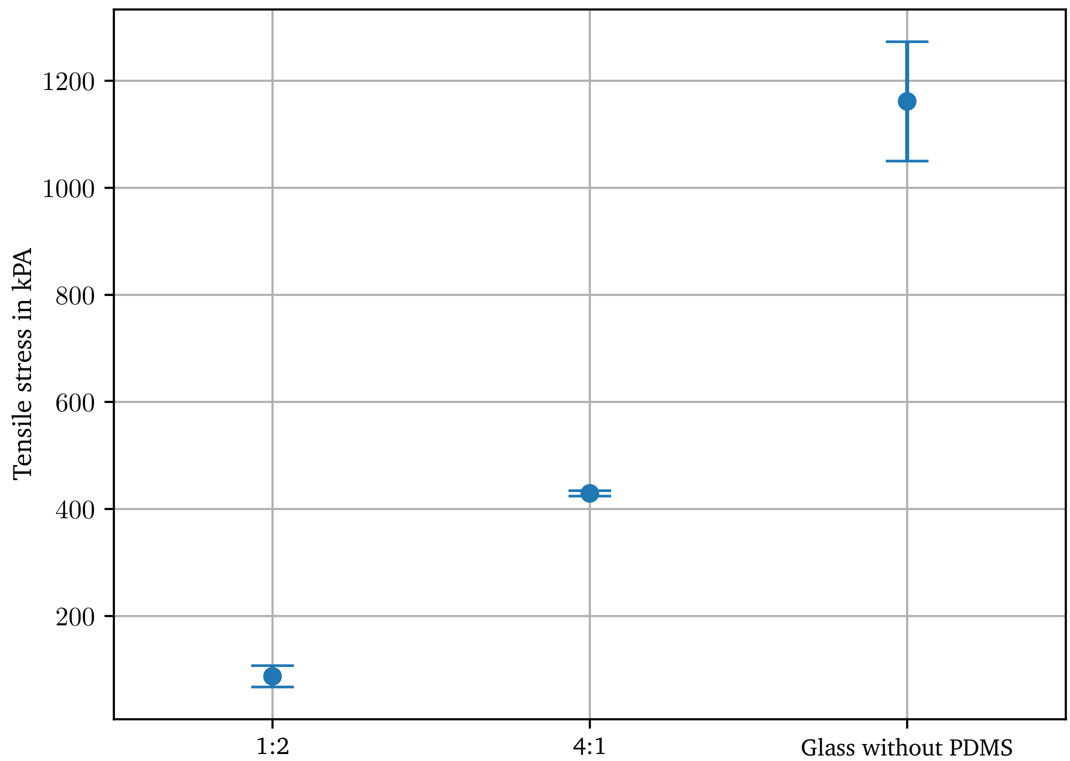


Figure 3.5: Comparison 4:1, 1:2 Base coat to curing Agent and glass without PDMS

In literature, the ice adhesion on PDMS without plasma treatment is $35\,\mathrm{kPa}$. for 2:1 and 5:1 the stress is between 60 to $80\,\mathrm{kPa}$ [15]. This is lower considerably lower than the experiment before. Therefore, one limitation is that the actual adhesion between ice and PDMS cannot be simulated by this experiment. Still, there is a correlation between the values and the experiment can give an insight of PDMS durability. In the end, if the separation happens between ice and pdms or pdms and glass are both good results.

In the next experiment, the effect of plasma curing is investigated. As the mixture ratio of 1:2 has the lowest adhesion, this experiment used this pdms mixture ratio. The same setup is used. Samples with a 2:1 weight ratio PDMS are additionally plasma treated before quickly clamping on the pulling machine. Even with low repetition rates, a clear tendency can be observed. With lower and stronger plasma treatment, the durable the PDMS Layer gets (Fig. 3.7). Over the whole range, The needed stress sextubles. Because the repititon rate is low, the exact values should be treated cautiosly. Also the results are not applicable to other mixture ratios, as different behaviour in plasma activation was observed between 2:1 and 4:1 weight ratio. also no glass-like state was observed in 2:1 weight ratio mixture.

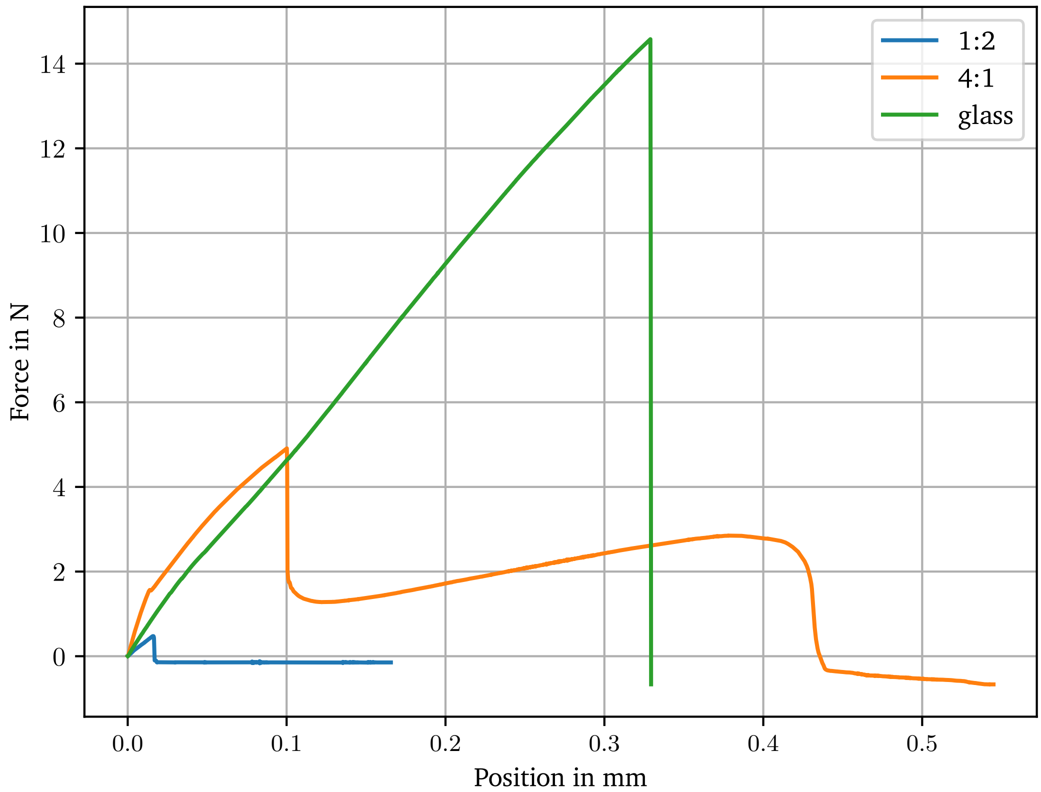


Figure 3.6: force over Time

As PDMS is hydrophobic, plasma activation is needed to freeze a thin layer of ice onto the coated slide. still, the lowest setting in the plasma machine(???) is not neccessarily enough plasma activation to get a low enough plasma angle. In a small test, PDMS coated slides with mixture ratio 1:2 is plasmaactivated with 25% for $0.1\,\mathrm{min}$, 30% for $0.2\,\mathrm{min}$ and 35% or $0.3\,\mathrm{min}$. 25% for $0.1\,\mathrm{min}$ does not deliver a low enough contact angle. 25% for $0.2\,\mathrm{min}$ contact angle is already very low. 35% with $0.3\,\mathrm{min}$ is definetly low enough. Both 25% for $0.2\,\mathrm{min}$ and 35% with $0.3\,\mathrm{min}$ are used in experiments.

PDMS wie aussehen in Raumtemperatur

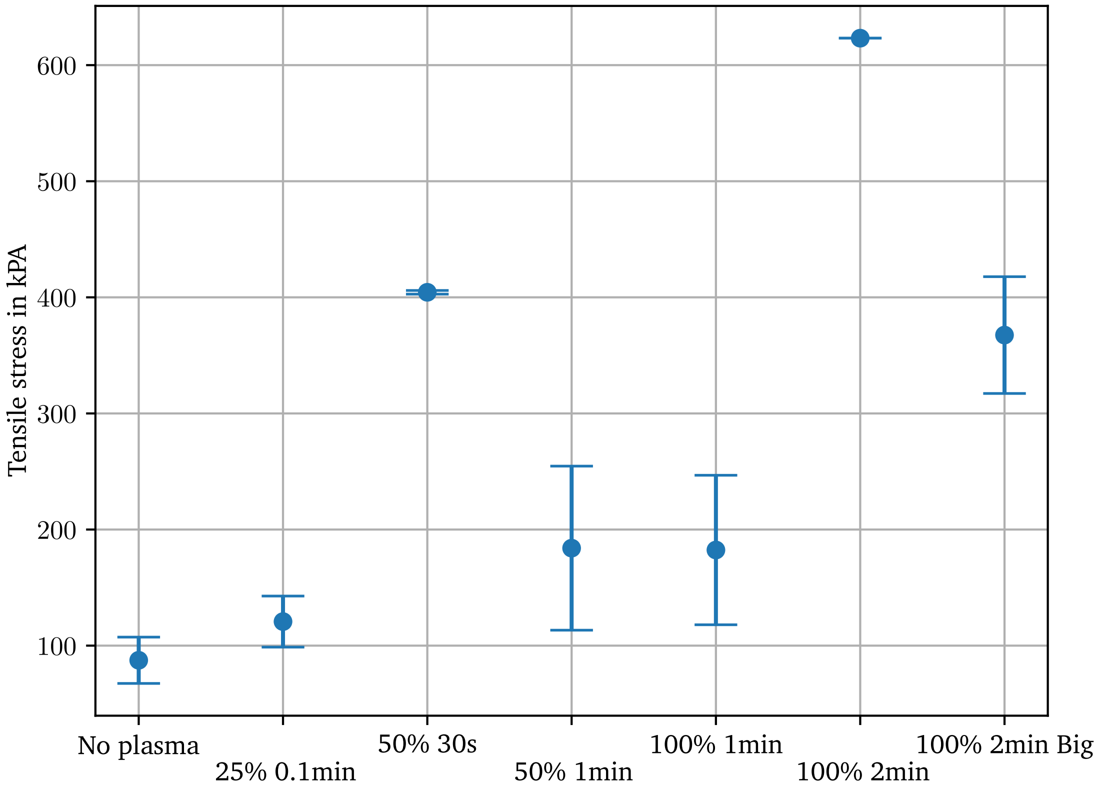


Figure 3.7: PDMS 2:1 Comparison between various Plasma curing strengths and durations.

3.3.2 experiments at cryogenic temperatures

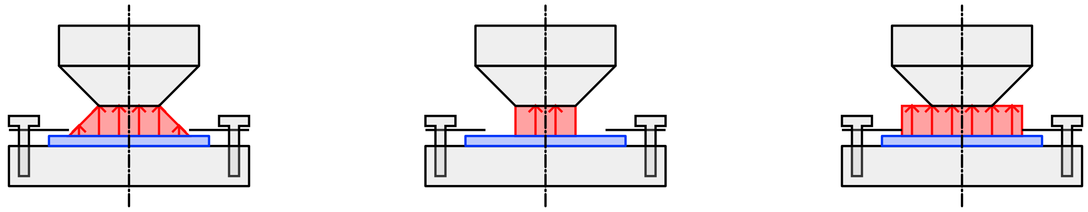
At cryogenic temperatures, three different mixture ratios are tested. 1:2 mixture ratio is used with minimal plasmaactivation, based on the result in previous chapter. 4:1 is used with 10 minutes 100% plasma activation. 50:1 is used with 3 minutes plasmaactivation with 25% and 100%. additionally for 50:1, plasma activation for 10 minutes at 100% is tested

1:2

To additionally measure the force applied of the finger, the pulling machine is used in combination with the finger. In the lab, the small bath is used for sample preparation. after microscopy, the sample is transported to the pulling machine. the big bath is fixed on the bottom of the pulling machine. on top, the finger is clamped into the shackle and aligned to the shuttle. The process is still as previously stated.

This setup has a lot of limitation. Aligning the finger is very difficult, as the screws are hard to reach in cold temperature. When cooling, the previous alignment ist lost as the coldness distort the setup. Also little movement at the finger has maximum effect on the sample and alignment. touching the tubes can loosen the sample and/or the setup needs to be new aligned. Even a bigger window is not enough. Errors in alignment and therefore gluing to the border of the window and loosening through movement are the two biggest error sources in this setup. So the old setup is used in further experiments to eliminate big error sources.

Over all pulls, the HFE can transfer a maximum force of $1.30 \pm 0.49\,\mathrm{N}$. The area which experienced the force is unknown. Additionally, the force distribution is uneven (e.g. like Fig. 3.8a). Still, a worst case and a best case which are easily calculated can be determined. as best case, all force is evenly distributed under the area of the finger (Fig. 3.8b). With this assumption, the Area is equal to the finger tip area. The worst case is an even distribution over the whole surface (Fig. 3.8c). The surface is clamped by the window. So the inner diameter of the Window is the worst case area. In reality, the Force is somewhere in between (Table 3.4).



(a) (b) (c)

Figure 3.8: Areas Vgl (TODO BESCHREIBUNG)

Area used	Area Size	Tensile Stress
Finger	$2.217\,\mathrm{mm}^2$	$585.4 \pm 219.4\,\mathrm{kPa}$
Small Window	$4.91\,\mathrm{mm}^2$	$264.3 \pm 99.1\,\mathrm{kPa}$
Big Window	$12.56\,\mathrm{mm}^2$	$103.3 \pm 38.7\,\mathrm{kPa}$

Table 3.4: different Areas used estimation of finger. The Real value is between the optimum of the finger area and the worst case of the window.

GRAPHIK HIER MIT MAxIMALER KRAFT DIE MIT HFE AUSGEWIRKT WIRD

RECHNUNG WIE DIE ZUGSPANNUNG IST ZWISCHEN DEN FENSTERN.

Zugmaschine mit Finger

4:1

50:1

4 Conclusion

In Experiments, no lipid solvent combination was found with high solubility at cryogenic temperatures. All tested solving processes are endothermic. In general, endothermic processes are inefficient in cold temperatures. To engineer a sacrificial layer, other detergents could be used [17]. An exothermic process is also not limited through the cyrogenic temperatures.

Detaching an ice layer from a lipid layer is not reliable

Different factors were examined on the effect of detaching of the finger.

At room temperature, the PDMS with different mixture ratios were tested. A mixture ratio of 1:2 has shown low adhesion forces as predicted. However, the transfer of results at room temperature to ice adhesion at cryogenic temperatures is only possible with restrictions. Plasma activation increases the adhesion forces potentially more on ice than the UV glue.

Generally, the "finger" is still not reliable after investigating the different factors and applying the insights. Additionally, issues with other assemblies like temperature controller and microscope reduce reliablity beforehand.

4.1 Outlook

The potential for lipids and detergents is not exhausted in this thesis. lipids and detergents can be engineered for lower adhesion forces. Additionally, finding solvents by solely experiments is very unlikely.

The force applied of the "finger" has proven to be not enough to break any surface on the sample. Pre broken pieces are sometimes picked up. A process is needed to completely loosen the ice layer without the "finger". For example, deactivating the plasma by putting a grid on the PDMS after Plasma activation could result in a loose, incontinuous but regular layer. Smaller pieces with less adhesion are easier to detach.

Another way to create a not continuous layer is with an imulsion.

Engineer the ice layer itself

Other ways than the finger should be tried to loosen the ice layer

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