Thin-Section Microtomy of Polymers for Microscopic Analysis

Petrik Blåfield

Process- och Materialteknik, 2018

EXAMENSARBETE	
Arcada	
Utbildningsprogram:	Process- och Materialteknik
Identifikationsnummer:	17513
Författare:	Petrik Blåfield
Arbetets namn:	Thin-Section Microtomy of Polymers for Microscopic Analysis
Handledare (Arcada):	Stewart Makkonen-Craig
Uppdragsgivare:	Stewart Makkonen-Craig

Sammandrag:
Syftet med detta examensarbete är att göra en metod för yrkeshögskolan Arcada att producera tunna tvärsnnitt av formsprutade samt 3D-printade delar. Ett tunt tvärsnitt är en 10-40 µm tjock bit av ett material. Tvärsnittet används i fel, material samt komposition analys. Ett optiskt mikroskop används för att analysera tunna tvärsnitten.

Medan det finns några olika metoder för att skapa tunna tvärsnitt, så koncentrerar detta examensarbete på att skapa dem med en mikrotom maskin. En mikrotom maskin skär med en kniv en tunn sektion av material från en prov bit. Medan mikrotomer också används för att skära tvärsnitt att biologiska och geologiska prov bitar, så används den producerade mikrotomen bara för olika polymer material.

Mikrotomi prosessen är relativt simpel. En provbit som kan vara av olika storlekar, blir fastsatt i provbitshållaren. Sedan skärs tvärsnittet loss med mikrotom kniven, som kan vara av olika material, men oftast av glas eller stål. Efter att tunna tvärsnittet är skuret, är den klar att läggas på ett mikroskop glas och sedan kan den analyseras med ett mikroskop.

För detta examensarbete, producerades två olika mikrotomer. En simpel handmanövrerad, som består av ett POM handtag, och ett höghastighetsstål blad. Andra mikrotomen som producerades använde samma stålbad, men skärande styrdes av en pneumatisk motor.

I detta examensarbete, jämförs mikrotomi också mot polering, som är en annan metod att producera tunna tvärsnitt.

Nyckelord:	Pneumatik, mikrotom, tunna tvätsnitt, höghastighetsstål, mikroskop,
Sidantal:	78
Språk:	Engleska
Datum för godkännande:	

DEGREE THESIS	
Arcada	
Degree Programme:	Process- och Materialteknik
Identification number:	17513
Author:	Petrik Blåfield
Title:	Thin-Section Microtomy of Polymers for Microscopic Analysis
Supervisor (Arcada):	Stewart Makkonen-Craig
Commissioned by:	Stewart Makkonen-Craig

Abstract:

The aim of this thesis is to produce a method of obtaining thin sections of polymers for Arcada.

A thin section is a 10-40 µm thick piece of material cut from a sample. The cross-section is used in failure, material and compositional analysis. The samples are analyzed using an Optical microscope.

There are different methods of creating thin sections, and this thesis concentrates on creating them using a microtome machine. A microtome machine uses a glass or hard steel blade to cut the thin section from a sample mounted in to sample holder. Microtomes can also be used to study geological and biological samples, but the microtome fabricated for the thesis, is used only for polymeric materials.

The microtomy process is straight forward, and after the sample is cut it can be mounted on to a microscope slide instantly, and then the sample is ready to be analyzed with an optical microscope.

For this thesis, two different microtomes were produced. The pilot microtome is a simple hand operated microtome with a polyoxymethylene with a high-speed steel blade. After testing, the microtome evolved to a machine with a pneumatic actuator for added stability and precision.

The microtomy method is compared to polishing, which is another method of producing thin sections.

Keywords:	microtome, microscope, thin section, injection moulded, optical analysis
Number of pages:	78
Language:	English
Date of acceptance:	

CONTENTS

ACKNOWLEDGMENTS

I would like to thank Stewart Makkonen-Craig, who has been an excellent and supportive supervisor for my thesis. He has provided constructive feedback and has been an excellent source of knowledge within failure analysis and optical microscopy.

I would like to thank Erland Nyroth, who's been a huge help when it came to fabricating the parts i needed for the microtome. Erlands expertise in different manufacturing methods was invaluable.

I would also like to thank Oscar Tulander, who worked parallell with me on his own thesis. He was a tremendous help to me as he provided many of the pictures I used in this thesis. He also helped me with information about the polishing method.

I would like to thank Pekka Blåfield at Etra Oy who provided loads of different samples to be tested with the microtome, along with help with sourcing some of the parts needed for the microtome. Harry Keskvaara at Isevat Oy was also a huge help as he manufactured the blade holder when they couldn't be done at Arcada.

Also a big thank you to Mikael Helenius, who provided a big help by checking the grammar and language of the thesis.

LIST OF ABBREVIATIONS

TEM	transmitted electron microscopy
SPM	scanning probe microscopy
SEM	scanning electron microscopy
OM	optical microscopy
DIC	differential interference constrast
POM	polyoxymethylene
PTFE	polytetrafluoroethylene
UHMWPE	ultra-high molecular weight polyethylene
PP	polypropylene
PBT	polybutylene terephthalate
µm	micrometer
$T_g s$	glass transitioning temperature

1 INTRODUCTION

The subject of this thesis is the construction of a microtome to obtain thin sections from injection moulded and 3D-printed parts and optical microscopy analysis of said parts.

A thin section is a 10-40 µm thick piece of a material; the thin section is used in failure, composition, and material analysis.

While there are multiple methods to obtain thin sections for optical microscopy analysis of polymers, using a microtome machine is regarded as the most straightforward. The microtome machine cuts the thin section from a sample with one cut, and the thin section is mounted on a glass slide and the sample is ready for analysis. Microtome machines are also very popular in preparing biological samples for microscopic analysis

There is a wide range of simple and complicated microtome machines available commercially. A microtome can be a simple handheld cutting device with a vise, or a large and expensive device with an oscillating blade.
When reviewing the commercial devices available and taking into account the budget and time constraints of this thesis project, it was decided that a microtome would be fabricated using in-house equipment and materials, to keep the costs as low as possible, and so that the microtome would be suitable to the needs of the school.

While microtomy can sound simple, there is much thought, precision and science behind the machine. Microtomy is thought of as the preferred method for preparing samples for optical microscopy, because in addition to its relative simplicity as microtomy does not modify the polymer thin section to any great extent.

The microtomy process starts with obtaining the sample which needs to be cut. The sample is restricted by size, but a wide array of different materials are suitable for microtomy. Mostly very hard and brittle polymers, thermosets and composites are not suitable for microtomy. Thin sections can be obtained from these materials using another method called polishing.

1.1 Motivation

1.1.1 Need for a Microtome Machine

Arcada's laboratory, while well-equipped was still lacking in some failure and material analytical infrastructure, which is important especially as Arcada produces numerous different materials and products. Arcada has an excellent optical microscope, which can be used to do optical failure analysis, but there was no way of obtaining the thin sections needed for analysis. The logical step was to make a machine that cuts thin sections which can be analyzed with the microscope.

1.1.2 Decision to Construct Microtome

High quality microtomes are generally meant for biological samples, which are usually softer than polymer samples. These microtomes could most likely be used for polymer samples, but their price was well above the budget for this project. There are cheaper, hand operated microtomes on the market, but it was found that it was very difficult to get uniform thin sections when cutting by hand.

The decision to construct the microtome came because the ones on market did not fit the budget, or the schedule. Also, because the majority of the microtomes on the market were not designed to cut hard polymers they would need a very stable base and sturdy blade.

1.1.3 Thesis Plan

The aim of this thesis is to find a method to consistently produce thin section for material and failure analysis. There are different methods of producing thin-sections and microtomy is one of the most used one, and the one that produces the fastest results. So, to achieve the aims it was the decided to construct a microtome machine which would be precise enough to cut thin sections which could be analyzed with Arcada's microscope. The aim was that the microtome would be used to produce thin sections from multiple different polymers and materials.

The plan was also to make comparisons to polishing, which is a different method to obtain thin sections.

1.1.4 Structure of Thesis

After the introduction, the thesis is divided into six different chapters. The second chapter is a literature review about microtomy, including general applications of microtomy, and how microtomy compares to other methods. The second chapter also includes information about sample preparation and the blades used in microtomy. The third and fourth chapters are both about microtome machines constructed for this thesis. Including the design process, material selection, fabrication and assembly of the parts. These chapters also include information about different issues that occurred with the microtome, and how they were fixed. The fifth chapter is about the experimental part, which includes the steps how to use the microtome, starting from sample preparation and finishing with the analytical techniques used. The fifth chapter also contains information about testing parameters and interpretation of the results.

The sixth chapter is about comparing microtomy to other methods such as polishing samples, and how the methods can be used together. The seventh and final chapter is about conclusions, containing a summary of work done, and discussion if the original aims were achieved. The chapter also contains recommendations on how to improve or modify the microtome, and which were the most suitable applications for it.

2 MICROTOMY

2.1 Introduction to Microtomy

Thin sectioning and microtomy are very old techniques. The microtome machine was invented in 1865 by Swiss scientist Wilhelm His, who also created the science of histogenesis. The device he invented was described as “A mechanical device used to slice thin tissue sections for microscopic examination” (The Editors of Encyclopaedia Britannica, 2012). Alongside polymer and biological studies, thin sectioning has also been used in metallurgy to study the microstructure of metals to determine their physical and mechanical properties used in failure analysis (Rosato & Rosato, 2000).
Microtoming is the most common method to formulate samples for optical microscopy, and it is a popular method, because if done correctly it does not damage or modify the polymer morphology to almost any extent

The process of microtoming is to obtain thin sections 10-40 µm thick of a polymer sample, to be analyzed optically with a microscope. Various sources state different optimal thicknesses of thin sections, but the general stated range is 10-40 µm. Thinner does not mean better, as the information the analyst seeks from thin sections cannot be found if the sample is too thin. The sample can be of many different materials with different material properties. Depending on the material properties of the sample, the cutting parameters can be altered (Scheirs, 2000)
Different cutting parameters that should be taken into account are, depending on what kind of microtome used:

- Cutting angle (between 15º-45º, depending on the material)
- Cutting speed
- Cutting force
- Blade

The quality of thin sections obtained by microtoming differs drastically depending on the material of the polymer. Soft polymers easily deform during the microtomy process and faults such as chatter marks can arise. Hard and brittle polymers can deform if the cutting angle is incorrect. Hard polymers can be softened or swelled with a solvent and soft polymers can be embedded in epoxy for example. Some very brittle samples for example may not be suitable for microtomy. For these sort of samples, polishing can be used to obtain thin sections.

Microtoming is an especially useful for stress and failure analysis. For example, analysis of a thin section is an excellent way of determining how the internal stress is distributed between the outer surface and interior of the plastic. Thin sections can be used to study properties of the polymer that vary based on different location in the cross section or across a parts thickness such as crystallinity, crystallite size, degree of dispersion of ingredients, residual antioxidant content, and degree of oxidation. The thin section can be used for infrared spectroscopy or optical microscopy (Erzin, 2013)

When a suitable sample material is obtained, it is mounted into the sample holder which is a precision vise. The height of the sample to be cut is then regulated in the vise so that a thin section with a suitable thickness can be cut. The fabricated microtome uses a pneumatic actuator to make the cutting maneuver. The cutting blade is a high-speed steel blade, mounted on a changeable self-lubricated nylon blade holder, which was designed specifically for the microtome. When the thin section is cut, the thickness is measured using a micrometer. A frequent problem before mounting the sample is that the sample tends to curl up, but this can be remedied with simple steps such as cementing the thin section on the microscope slide. When the thin-section is uniformly mounted on the microscope slide, it can be analyzed with a microscope.

2.1.1 Overview of the Microtoming Process

Microtoming is a relatively straightforward process with only a few steps. Microtoming starts with getting or selecting a sample that will be analyzed. The sample can be almost any polymer, but semi-crystalline polymers work best. For example, polyoxymethylene (POM) or polyethylene (PE). With thermoset polymers, some issues may occur. After the sample is selected, the next step is to select the blade and the cutting angle. Normally a hard-steel blade is used to obtain thin section from polymers. A glass blade can be used to obtain ultrathin section (0.1-10 µm). The angle of the blade is the next thing to consider. The angle is selected based on the material of sample, and the angle is always between 15º and 45º. The general rule is that the harder the sample, the higher the blade angle. If the sample is within the size constrains of the microtome, and the sample is not too soft or hard, it can be directly mounted on to the sample holder. If the sample is too large, it can be polished, or cut into a suitable size using a band saw. If the sample is too small or soft, the sample can be embedded in epoxy or another embedding resin.

The sample must be tightened rigidly on to the sample holder, but if there is too much pressure on the sample it may deform. Depending on the microtome used, to get the desired thickness of the sample either the sample holder or blade has to be adjusted. The thickness of thin section should be between 10-40 µm. If the section is too thin the desired information may not be provided. When the sample is mounted, its free portion should not exceed 2 mm, as the cutting may deform the sample.
When the sample is properly mounted, and its height adjusted, the cutting procedure can begin. On the microtome produced and used in this text, the cutting speed and cutting force can be adjusted, and it should be adjusted depending on the material properties of the sample.

When the thin section has been cut it should be placed on a microscope slide using tweezers. The sample must be flat on the microscope slide. If the sample curls up which is the most common problem, it can be glued on to the slide using Canada balsam, or placed on the surface of heated water to straighten it up.
When the thin section is straight and uniform on the slide, the thin section is ready to be analyzed.

2.1.2 Applications of Microtomy

The thin sections obtained by microtomy have numerous different applications in failure analysis, quality control and troubleshooting.
Depending on what information is needed, the thin sections should be cut from various places on the sample. For example, if examining injection molded products, the most information about the molding process can be derived if the sample is cut near corners or gates (Scheirs, 2000). If information about the cooling history about the sample is wanted, it should be cut in a cross-sectional manner, and the spherulitic structure should be studied (Scheirs, 2000).

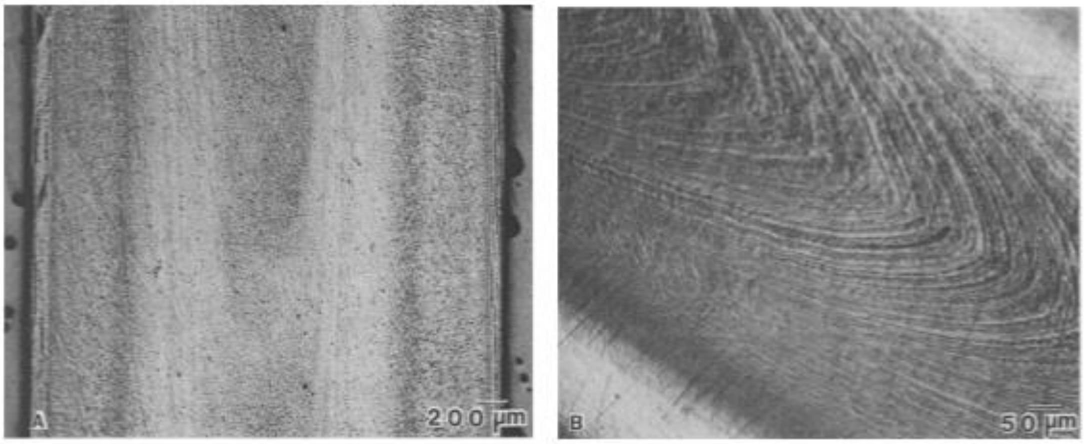

Figure 1: Using reflected light to flow patterns in a thin section of an extruded sample. (left). Using DIC to show the flow pattern in greater detail (right) (Sawyer, et al., 2008)) .

Thin sections obtained by microtomy can be used in multiple different microscopy methods, such as Optical microscopy (OM), scanning electron microscopy (SEM), transmitted electron microscopy (TEM) and scanning probe microscopy (SPM).
OM is the most common way to analyze thin sections, and the same method can be used to obtain samples for TEM and SPM.
OM reveals a vast amount of different information on the thin section, depending on what illumination technique is used. Figure 1 shows how using reflected light and differential interference contrast (DIC) shows the flow patterns in sample in two different ways.

Microtomy can be used for SEM also. SEM is used to study surfaces of different polymeric materials such as plastics, fibers, membranes and composites. The outer surface can be studied, alongside fractured samples. A conductive coating is applied to specimens being studied with SEM, typically by sputtering a metal thin metal layer or by carboncoating for elemental analysis (Sawyer, et al., 2008).

2.1.3 Applications of Ultramicrotomy

Ultramicrotomy is the preparation of ultrathin sections of polymeric materials for observation in an electron microscope. For ultramicrotomy a glass or diamond blade is usually

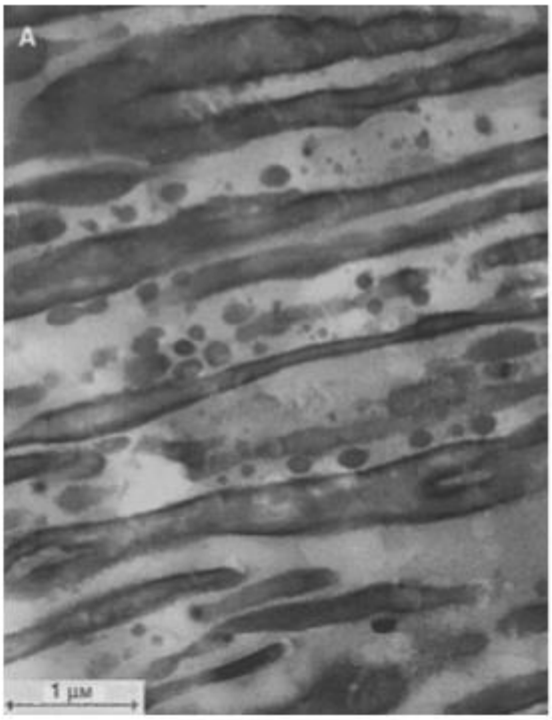

Figure 2: Ultrathin section of polypropylene, sectioned at room temperature using a 35º cutting angle and a diamond blade. (Sawyer, et al., 2008)

used, as steel blades do not provide the same accuracy. The thickness of ultrathin sections is approximately 30-100 nm. Ultramicrotomy is also common with biological samples.

2.1.4 Sample lighting techniques for optical microscopy

Even a simple optical microscope can be used in various different ways by changing the way the sample is illuminated. Scheirs states the following as the most used ways of illumination (Scheirs, 2000):

- Transmitted light
- Near-vertical illumination (light falling at an angle between 80º and 90º)
- Incident illumination (light falling at an angle between 30º and 80º
- Grazing angle illumination (light falling at an angle between 0º and 30º)

2.1.4.1 Transmitted Light

Transmitted light is normally used to determine how the pigments and fillers have been distributed in a thin section along with finding voids and impurities. A polarizing filter can be highly useful when using the transmitted light technique as rotating the sample or the analyzer, as it shows different inclusions in the sample.

2.1.4.2 Near-Vertical Illumination

Mainly used to light voids, holes and deep pores in a thin section. Fiber-optic microscope lights are very useful when using this method.

2.1.4.3 Incident Illumination

Incident illumination is very good when used to highlight fractured surfaces and offers contrasting features. Fiber-optic microscope lights are useful for this method and adjusting the lights can increase or decrease the contrast on the specimen surface. This method can produce misleading artifacts on the thin sections, mainly shadows.

2.1.4.4 Grazing Angle Illumination

Grazing angle illumination is used to view the flow lines and flow patterns mainly in injection molded thin sections. It can also be used to find the location of weld points.

2.1.4.5 Other Illumination Techniques

There is a large number of different illumination methods used depending on what information is needed. Coaxial illumination is used to emphasize irregularities or to reveal graininess on flat specular surfaces, used for reflective polymers.

Dark field illumination is a technique that involves illuminating the thin section on one side with a fiber optic light, in such a way that no light reaches the microscope objective directly. Cracks and other flaws on the surfaces scatter the light and appears bright and uniform areas remain completely dark. Most defects and fillers can be observed with this method.

Another method is phase contrast which involves converting the different phases is in light, which are not visible, into observable visible phases. This means that on the specimen all details that diffract light appear dark on a bright background. This illumination technique is used to examine the microstructure of polymer blends as different blend parts have different refractive indices. However, only ultrathin (1-3 µm) sections can be used for this method. (Scheirs, 2000)

2.1.4.6 Polarized microscopy

It is very simple to use polarized optical microscopy, as it works by placing a polarizing filter below the condenser on the microscope, and another filter above the specimen. The filters must be at a 90º angle from each other so that the polarizing effect works.

Polarizing the sample allows for analysis of the cooling rate, thoroughness of mixing, inclusion as well as various defects such as weld lines. Information can be found about the specimens processing conditions by studying the spherulitic microstructure. Polarized microscopy is also used when examining fiber-reinforced semi crystalline polymers specimens. (Scheirs, 2000)

The thickness of the sample being analyzed depends on the material. Polypropylene (PP) and polybutylene terephthalate (PBT) have very fine spherulitic structures, and therefore very thin samples are required, usually thinner than 5 µm.

Scheirs (2000) states that the ideal thickness for this kind of sample is between 1-2 µm, and in samples thicker than 5 µm interference colors may appear (Scheirs recommends ultramicrotomy when the aim is to study the spherulitic structure of these polymers).

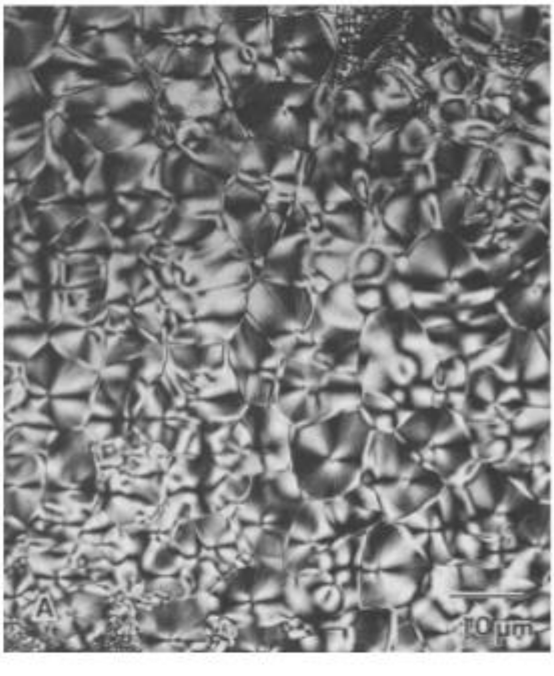

Figure 3: A thin section of nylon, illuminated in polarized light, revealing a coarse spherulithic texture. (Sawyer, et al., 2008)

2.1.5 Advantages of Microtomy - Compared to Other Methods.

The greatest advantage of microtomy is its speed and ease of use. With a microtome a thin section can be ready for analysis in about 10-20 minutes, including all steps of preparation. Depending on the size or materials properties of the sample some additional steps may have to be taken, such as embedding the sample if it is too soft or cutting down the sample if it is too large. But normally when cutting a semi-crystalline or other relatively simple polymer it is as easy as mounting the sample on to the holder, cutting it and then mounting the thin section a microscope slide.

2.1.6 Comparison to Polishing in the Literature

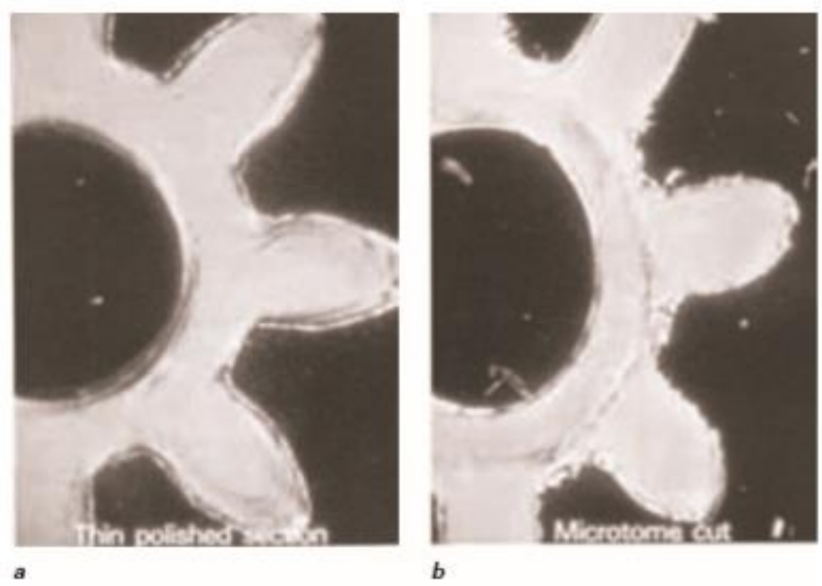

Figure 4: A comparison of a polished thin section (left) and a microtomed thin section (right). Notice that the polished thin section is not as deformed and looks sharper. (Böhme, 1990)

Polishing is alongside microtomy the most ued method of obtaining thin sections. The process of polishing is more complicated and time consuming when compared to microtomy but can offer better results when it comes to analyzing the thin sections.

The process of polishing starts with embedding sample in a resin. Böhme recommends using an epoxy or polyester resin, depending on the type of polymer being embedded. (Böhme, 1990). After the sample is embedded and the resin has hardened, which can take up to 12 hours, depending on the resin and hardener used, the sample has to be polished using a polishing machine. First one side of the sample is polished, and then that side is

cemented on to a microscope slide using epoxy or a glue. When the glue has hardened the other side of the sample is ground down using gradually finer and finer paper.
Scheirs states that when choosing the method to produce the viewing angle required, preparation time, and the nature of the component should be taken into account. (Scheirs, 2000). The biggest advantage of polishing when compared to microtoming is that polishing allows fractured samples to be analyzed, and at angles which are not possible with microtomy. Polished samples also do not show any signs of distortion, damage or stretching that microtomed samples can show.
While composites can be microtomed when using a saw microtomer or a diamond wire-saw, polishing is the recommended way as fibers may be torn when using a microtome. Scheirs continues that obtaining thin sections with either method is very challenging, as in a composite the resin or matrix material is removed more rapidly compared to the fibrous material. (Scheirs, 2000)

2.2 Literature Review of Microtomes and Microtomy Processes.

2.2.1 Sample Preparation and Microtomy Techniques

The procedure of preparing a sample for microtomy is simple but can have numerous problems. The most common problem is that the thin sections roll up, this especially happens if the polymer component has built-in stresses. A simple way to solve this problem is to press a piece of transparent adhesive tape on the surface of the specimen (this should be done before the thin section is cut from the specimen) and holding the other end of the tape with tweezers to stop the thin section from curling up. The thin section with the piece of tape could be transferred directly to a microscopy slide. This can however affect polarized microscopy as the tape is birefringent.
Another problem that can occur is that the thin section which is being cut remains partly connected to the specimen. If the thin section is pulled by force from the specimen it may result in deformation of the thin section. Scheirs (2000) recommends incising the tail of the sample before cutting.

When the specimen is mounted on to the microscope slide it must be totally flat. If the edges of the specimen roll up even a bit the microscope will not be able to properly focus on the specimen. There are a few different methods in keeping the thin section flat on the slide. One way is to use Canada balsam. Canada balsam is placed on the microscopic slide and then the thin section is placed on the balsam using tweezers, and then pressed on with preferably a wooden stick. After this a cover slip with a small amount of Canada balsam is pressed upon the specimen. If the microscope is still not able to sharply focus on the specimen, it means that the specimen is still not flat on the slide. The next step is to heat the whole specimen (along with slide) on a hotplate to 65 ºC and then press the specimen with a warm metal weight. This method is also good to remove air bubbles.

Scheirs (2000) notes that as Canada balsam contains xylene as a solvent, it cannot be used for all polymers. Paraffin oil can be used as a substitute but is has the downside of having low viscosity. All semi-crystalline polymers should be embedded in paraffin oil as it removes refraction at irregularities and sample edges, and that way removing artifacts. Paraffin oil cannot however be used for styrenic polymers such as ABS because of their refractive index.

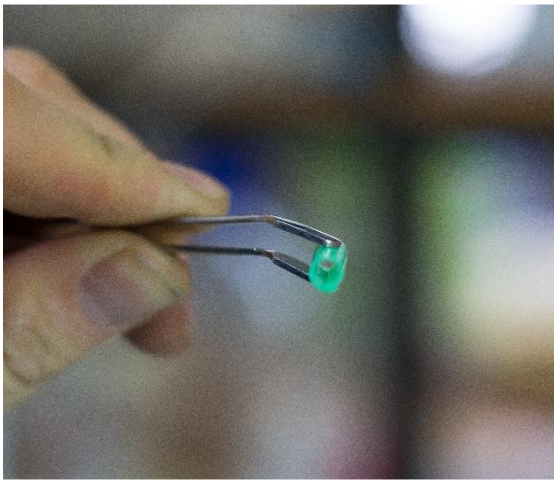

Figure 5: Example of how a thin section can curl up. The sample in the picture is ultra-high molecular weight polyethylene. The sample can be straightened gently with the tweezers, and then put under a weight. The sample could also be warmed in water. The sample curls up due to internal stresses in the sample. (Picture provided by Oscar Tulander, 2018)

Even if a thin section can be perfectly mounted, there can still be numerous issues with it. For example, if there are chatter marks on the surface of the thin section it can mean that the knife is too blunt, or that the tilt angle of the blade is too high or low. The chatter

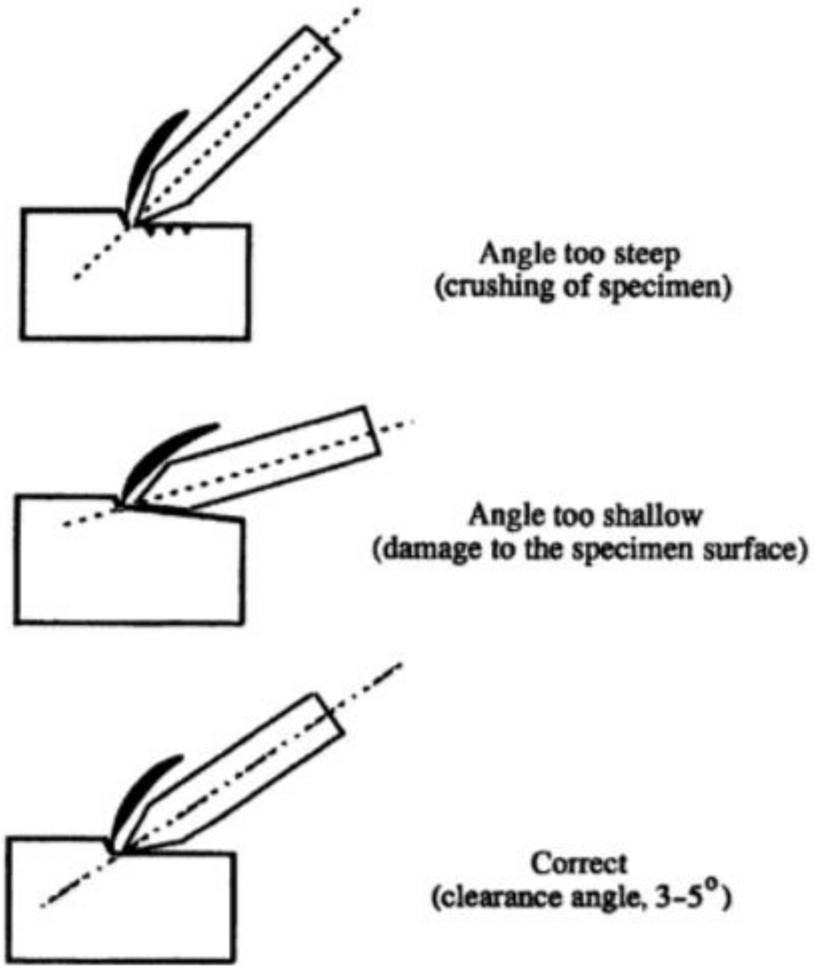

Figure 6: The effect of different cutting angles. If the angle is too high, it may crush and deform the specimen. If the cutting angle is too low, it can cause smearing. (Scheirs, 2000)

marks can also occur if the sample is a bit loose in the holder, if the sample is too large, or if the material of the sample is too soft.

If the thin section appears to be crumpled or uneven, there can be problems with the sharpness of the blade, cutting speed (too fast or too slow), or even the softness or hardness of the sample material.

If the thin sections tend to roll up, it can depend on the angle of the blade, or if there is orientation or stresses in the material. (Scheirs, 2000)

If the sample cannot be stabilized by cementing or embedding, Cryotomy may be the only solution. Cryotomy is when a sample is cooled down below their glass transitioning temperature ($T_g s$). The samples are cooled using dry ice or liquid nitrogen, and then embedded into a mixture of polyvinyl alcohol and water (if below -30 ºC). When frozen, this mixture should not be hard and brittle such as ice, but soft and firm (Scheirs, 2000). Sawyer (2008) states that cryomicrotomy is becoming an increasingly popular method to prepare samples for SPM and TEM.

According to Sawyer (2008), the following materials can be sectioned at room temperature, without any additional preparations:

- Polycarbonate (PC)
- PMMA
- HDPE
- Epoxies
- Nylons
- Polyurethanes (if rigid enough)
- HIPS (high impact polystyrene)
- Polypropylene
- ABS

Sawyer (2008) continues that the following material may need to be cut below their glass transitioning temperature (cryotomy), because they may crack or shatter when cut at room temperature:

- PP
- PE
- Elastomers
- PTFE
- PVC
- Latex
- Paints
- Silicones

2.2.2 Blades

The choice of microtome blade is a critical factor when seeking optimal thin section results. When cutting polymer materials, the most commonly used blades are hard-steel blades or glass blades.

Glass blades are produced by fracturing glass and are mostly used in ultramicrotomy, for samples obtaining samples with thickness from 1-10 µm. Fresh glass knives have such a sharp cutting edge, that they can cut different polymers where even the most high-quality steel blade would cause fine ripples in the slice. Glass knives are comparatively cheaper than steel blades and are usually wedge-shaped. Glass blades are not used for the micro-tome produced for this thesis, as the blades would have to be produced in house, and that would require a glass cutting machine. (Scheirs, 2000)

With the hard-steel blades, there are three commonly used different blades shapes, plano-concave, wedge-shaped or planing-blade shaped. Each different shape is used for different kind of material.

Scheirs (2000), states that the plano-concave shaped hard-steel blade with a sharp angle is used for soft foam materials such as polystyrene foam. The wedge-shaped blade is used for softer plastics such as polyethylene and rubbers. Planing-shaped blades are used for hard plastics such as POM, PVC or ABS, but the angle of the blade should differ depending on the material. Blades with tungsten carbide tips are recommended when cutting thin sections from POM or PA.
When cutting with a high-speed steel blade, care needs to be taken as the blades can flex or dig into the sample. The steel-blades can also leave surface scratches on the specimen.

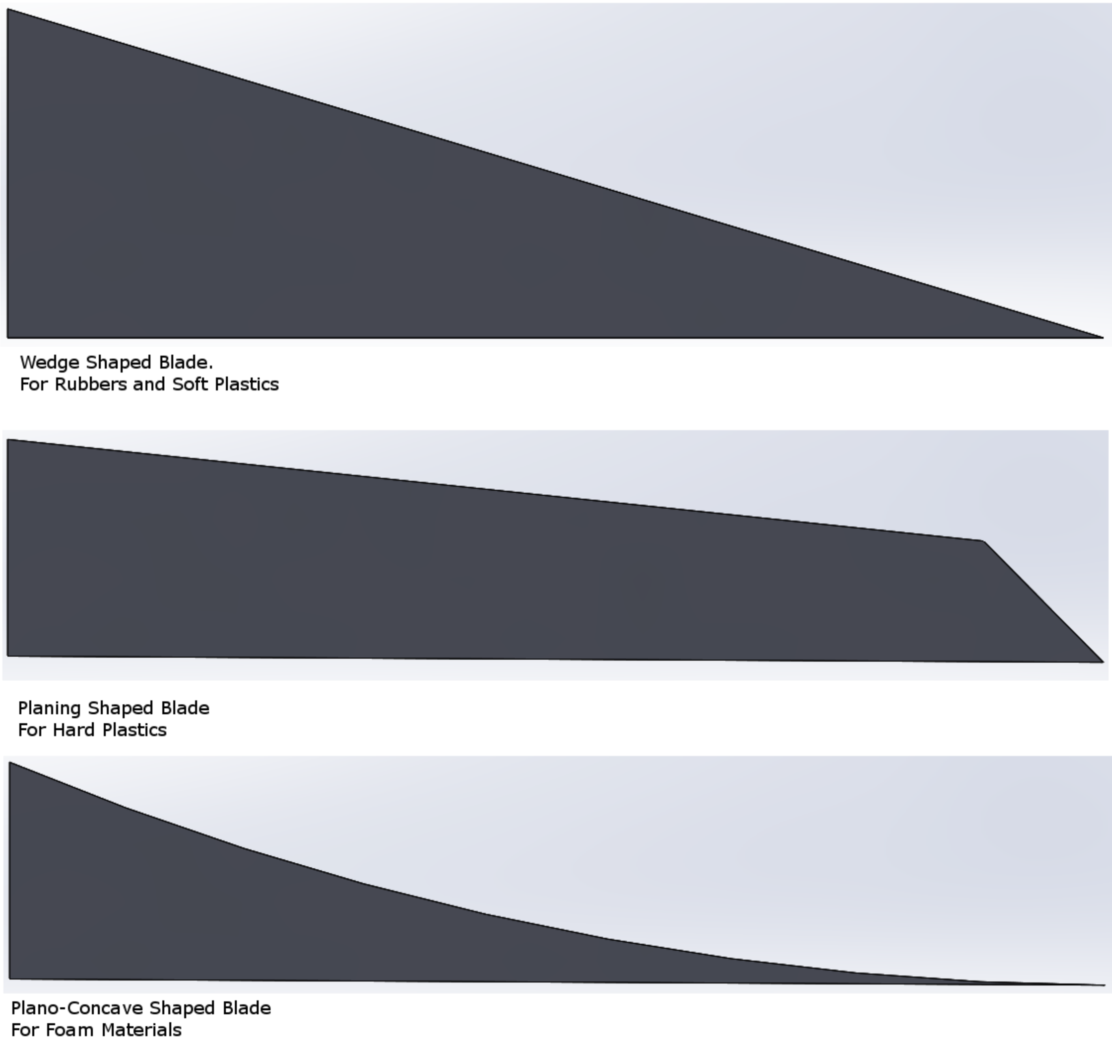

Figure 7: The most common shapes of steel blades used in microtomy, and what sort of polymer they are most suited for. Designed on SolidWorks.

2.2.3 Troubleshooting table

Table 1:A table with different issues that can occur with the thin sections, along with the potential reasons for the problem and potential solutions. (Scheirs, 2000) (Sawyer, et al., 2008)

Problem	Probable Reason	Solution
Thin section has chatter marks	Blunt blade, too high or low cutting angle, sample not fastened correctly.	Sharpen or change blade, adjust cutting angle, secure the sample again (check that the edges of the sample are even).
Crumpled or wrinkled thin section	Blunt blade, cutting force too high, cutting speed too high	Sharpen or change blade, adjust cutting force (from the pressurized air valve), adjust cutting speed (from the valves on the actuator)
Thin sections roll up	Cutting angle too high, stress or orientation on the sample	Lower cutting angle, use the tape method described previously.
Sample breaks, thin section unobtainable	Sample is too brittle	Use another method; polishing/grinding
Striations or lines in the section	Nicks or imperfections on the blade edge	Sharpen or change blade
Thin section not of uniform thickness	Cutting angle too low, specimen too hard.	Adjust cutting angle, re-trim the sample before cutting new thin section

3 PILOT HANDHELD MICROTOME

3.1 Aims

Initially the goal was to make the microtome as simple as possible, which meant that the microtome would be handheld, with just a blade attached, and something to hold the sample stable.

There is a large amount of different handheld microtomes on the market, with many of them being very simple holders for a razor blade. The commercial versions of handheld microtomes were largely meant for softer biological samples, so they were quite flimsy and would most likely not be able to cut harder or sturdier polymers.

The blades available for handheld microtomes were also quite flimsy, with the majority meant for biological samples. Glass blades were not considered for the handheld microtome, as there was not a method available for making the glass blades, and because they would be worn down very quickly. The blade would have to be hard and durable, but still affordable.

The sample holder is another important part of this microtome. The sample holder is used to guide the cutting maneuver, while keeping the sample steady and free of any movement.

3.2 Design

Before fabricating the handheld microtome, it was designed using SolidWorks. The design of the handheld microtome was made after acquiring the blades and sample holder which would be used, so the design would have to accommodate those parts.

The microtome would need to be sturdy so that it would not break when applying large amounts of pressure.

The shape of the microtome was to be a simple L-shaped structure, on which the top part was the handle, and the lower part would hold the blade. The blade would be fastened by screws.

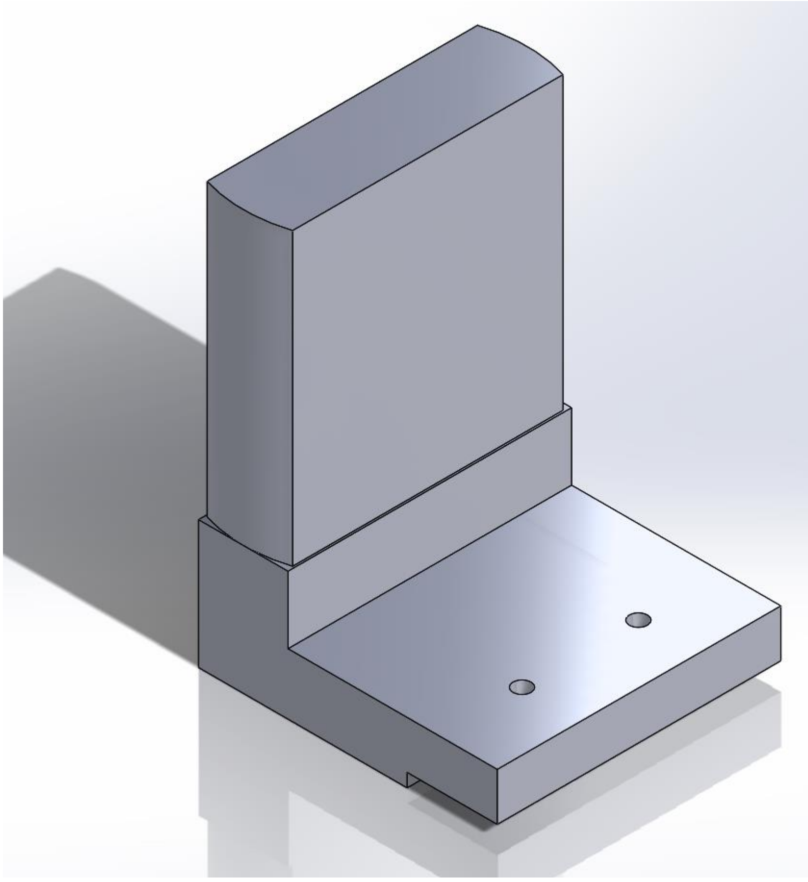

Figure 8: The design of the handheld microtome in SolidWorks.

3.3 Material selection

3.3.1 Microtome Handle

The material for the microtome had to fulfill a few different requirements: light but sturdy, easily available, and cheap and easy to machine.

The material chosen was polyacetal or polyoxymethylene (POM). POM is a semi crystalline engineering thermoplastic. The material has exceptional creep resistance, toughness and fatigue strength (Ibeh, 2011). POM is also has good ductility, which means it can be machined sawed or drilled without cracking.

Figure 9: The handheld microtome, made from POM, without blade.

3.3.2 Blade

An high-speed steel blade was chosen for the microtome that was constructed. The blade is specifically a Makita SKH K1C Planer blade. High-speed steel is a tool steel, named for its ability to cut and machine even hard materials at high speeds. There are numerous different classes of high-speed steels but they are all complex iron-base alloys of carbon, chromium, vanadium, molybdenum, and tungsten (Readon, 2010).

The blade used here was a class W5 High speed steel blade. The classes are based on what the steel composition contains. The composition limits for the W5 class are the following:

Table 2: The composition limits in W5 high-speed steel, in percent of mass. From left to right; Carbon, Manganese, Silicon, Chromium, Nickel, Molybdenum, Tungsten, Vanadium, Cobalt. (Readon, 2010)

C	Mn	Si	Cr	Ni	Mo	W	V	Co
1.05-1.15	0.10-0.4	0.10-0.4	0.40-0.60	0.20 Max	0.10 Max	0.15 Max	0.10 max	0

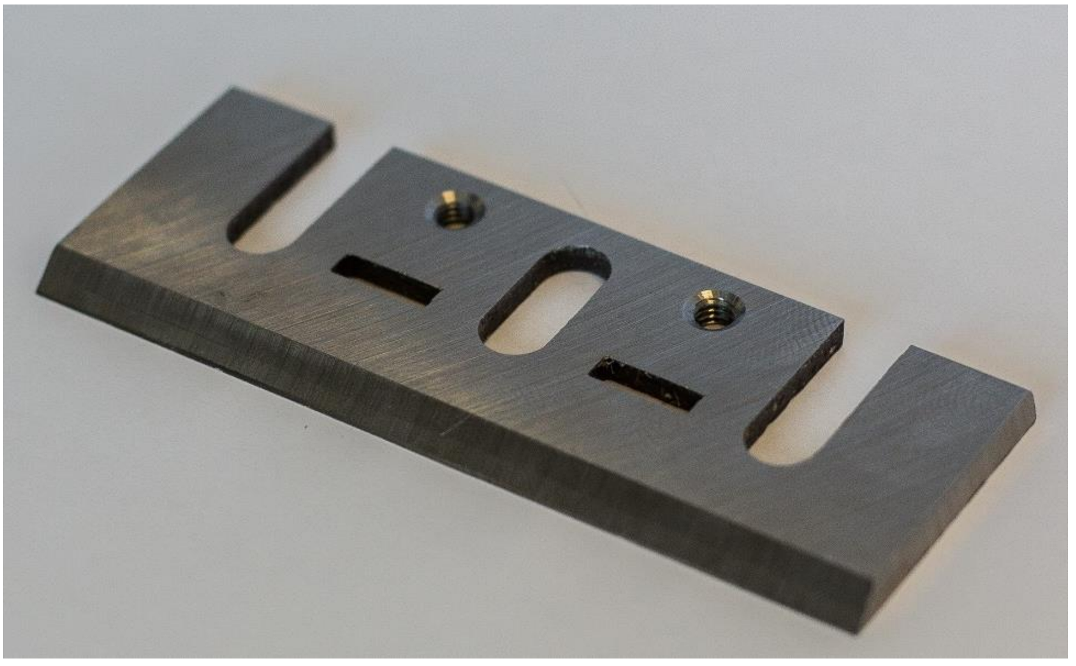

Figure 10: The Makita SKH K1C W5-HSS Blade used in the microtome. The blade has a cutting angle of 45º. (Picture provided by Oscar Tulander, 2018)

The group W steels are water-hardening steels. Water-hardening steels contain carbon as the main alloying element. Small amounts of chromium (0.40-0.60 %) are added to group W steels to increase their hardenability along with its wear resistance. Vanadium (0.10%) is added to maintain fine grain size, enhancing the toughness of the steel. W5 steel has 1.05-1.15 % of carbon content. As water-hardening steels have low resistance to softening at higher temperatures, they are most suitable for cold heading, striking, coining, wood-working tools, hard metal cutting tools and are also used as wear-resistant machine tool components. (Readon, 2010)

The blade used in the microtome can be changed, but as the sled that holds the blade is cut specifically for the Makita SKH K1C W5-HSS blade, only blades of the same size can be used. While the blade used is a planing-shaped blade, there are sleds with two different cutting angles, the plano-concave shape and a sled with a 45º cutting angle. Two of the blades were ground down to a 30º cutting angle. The blades could also be ground down to other angles, but generally all samples can be cut on angles between 15º-45°

The blades will lose their sharpness and wear down over time, and can be maintained with proper equipment, such as a sharpening stone. But as the blades are of such a hard material, they should last a relatively long time in normal use. The recommended course of action is to replace the blade with a new when the old ones are worn down, as they are cheap and durable, with a pair of new ones costing under 10€.

3.3.3 Sample Holder

The sample holder for the microtome needed to be sturdy and precisely made as the blade holder moves along the sample holder, with the sample holder acting as "guide". With the blade holder sliding along the sample holder, there cannot be any vertical wobble which could potentially ruin the sample.
The sample holder also had to be very versatile, so a wide array of samples with different material properties and sizes could be used.
The sample holder chosen for the microtome was a Xueling Precision vise, made out of 20 CrMnTi steel and hardened to a 56-58 on the Rockwell hardness scale. The vise is precision ground on every side, which is important as there cannot be any dents or imperfections. The parallelism and squareness precision on the vise is 0.5 µm.

Table 3: The composition limits in 20 CrMnTi steel, in percent. From left to right; Carbon, Manganese, Silicon, Chromium, Phosphorus, Sulfur (Otai Steel, n.d.)

C (%)	Mn (%)	Si (%)	Cr (%)	P (%)	S (%)
0.95-1.05	0.25-0.45	0.15-0.35	1.4-1.65	<0.025	<0.025

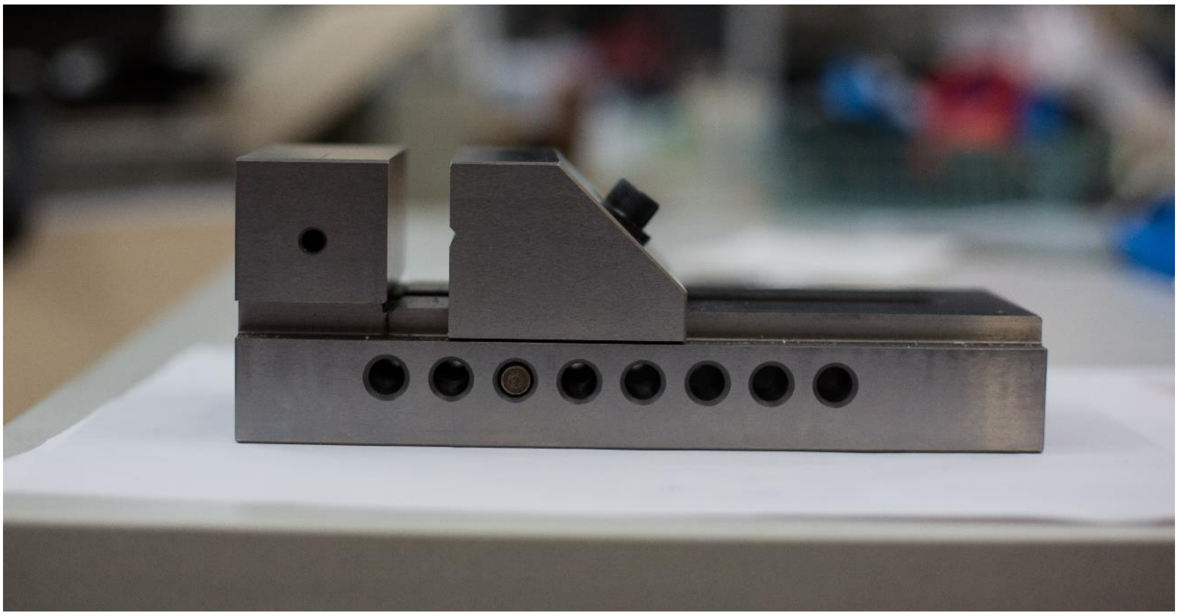

Figure 11: The Xueling Precision vise, used as the sample holder for both the handheld and pneumatic microtomes. (Picture provided by Oscar Tulander, 2018)

3.4 Fabrication

The fabrication of the handheld microtome was very straightforward. The handle was machined with a HAAS mill from suitable piece of POM. The recess on the lower part of the microtome was for the blade. The recess was the exact same thickness as the blade, so the blade and the bottom of the handle would be on the same level.
The handle was rounded out so that there would not be any sharp edges for user friendliness. All the machining routes were made in MasterCAM.

The handle was the only part that was fabricated for this microtome, as all the other parts had been bought and stayed largely unmodified.

3.5 Assembly

The assembly of the handheld microtome was really straightforward as only the blade needed to be fastened to the handle. The fastening hole on the blades were threaded, so the assembly was done with two screws and was then fastened to the handle. The screws had to be shortened as they could not come out on the bottom side of the handle.

3.6 Testing

The handheld microtome was tested on numerous different polymers. Including polycarbonate (PC), polypropylene (PP), polytetrafluoroethylene (PTFE), polyethylene (PE) and ultra-high molecular weight polyethylene (UHMWPE).

The samples used were first cut using a band saw so that the size was suitable for the sample holder. As the only restricting factor for the size was the sample holder, they could be quite large, so samples of various different sizes were tested.

After reviewing different microtomy techniques available in the literature, some were

Figure 12: The hand-operated microtome in use. The sample in the picture is PTFE. (Picture provided by Oscar Tulander, 2018)

tested in practice.

When using the hand microtome, there was no method of regulating the height of the sample.

After the sample was secured in the holder, the thin section is cut by simply pressing the microtome down and pushing it through the sample, like using a planning machine.

3.7 Analysis

The hand microtome had no problems with cutting the majority of materials. Even without using a proper method of regulating sample height, the hand microtome was able to cut sections of the sample materials. The hand microtome was very easy to use and the full process of mounting the sample and cutting the section only took a few minutes.
The thin sections produced were not consistent, but some were thin and uniform enough to be analyzed with the microscope.
The blade's 45º cutting angle proved to be very good for the majority of materials and had no problem in cutting into the different samples. Even after rigorous testing, the blade was not worn out at all.
The vise also proved to be a very good sample holder. The precision cut hardened steel surface allowed for the blade and handle to slide over it providing a steady surface for cutting. The sample also proved to be very versatile allowing sample of different sizes and shapes to be cut.

3.8 Conclusions for the Next Prototype

While the hand microtome was able to cut sections of from the samples, the thin sections were not consistent enough. The inconsistencies were due to a few various reasons.
The main reason was that as the microtome was operated by hand, it was difficult to apply a constant amount of force. As the force was inconsistent, it led to jagged, broken and uneven thin sections.
Another reason for the inconsistencies was that the sample holder was not secured to anything. This was an issue when cutting into the sample, the holder could move or wobble, which led to inconsistent thin sections.
With the blade, there were no issues. The blade could always be sharpened or modified so that the cutting angle would be 15º or 30º, but the 45º cutting angle was suitable for tested all tested materials.

The next prototype would have to have a different method of cutting, that would not be dependent on the operator's steadiness. This would lead to more consistent thin sections. The vise would have to fastened as there can be no room for it to move or wobble.

The blade had no issues with the sample materials, but there would be room for more versatility regarding the cutting angles if there were different methods of mounting the blade. There would also have to be a consistent method of regulating the height of the sample, as approximation leads to inconsistent thicknesses.

4 PNEUMATIC MICROTOME

4.1 Design Considerations

The construction of the pneumatic microtome was the biggest and most time-consuming part of this thesis.
The aim when designing the microtome was to construct a sturdy machine that is precise enough to cut the thin section between 10-40 µm, and simple to use to that the thin-sections could be made in 10-20 minutes.

The design of the microtome was ever evolving, and many different models were planned, and the design eventually evolved to the pneumatic actuator powered microtome. Failed designs, material availability and time constraints, all played a part in the final microtome.

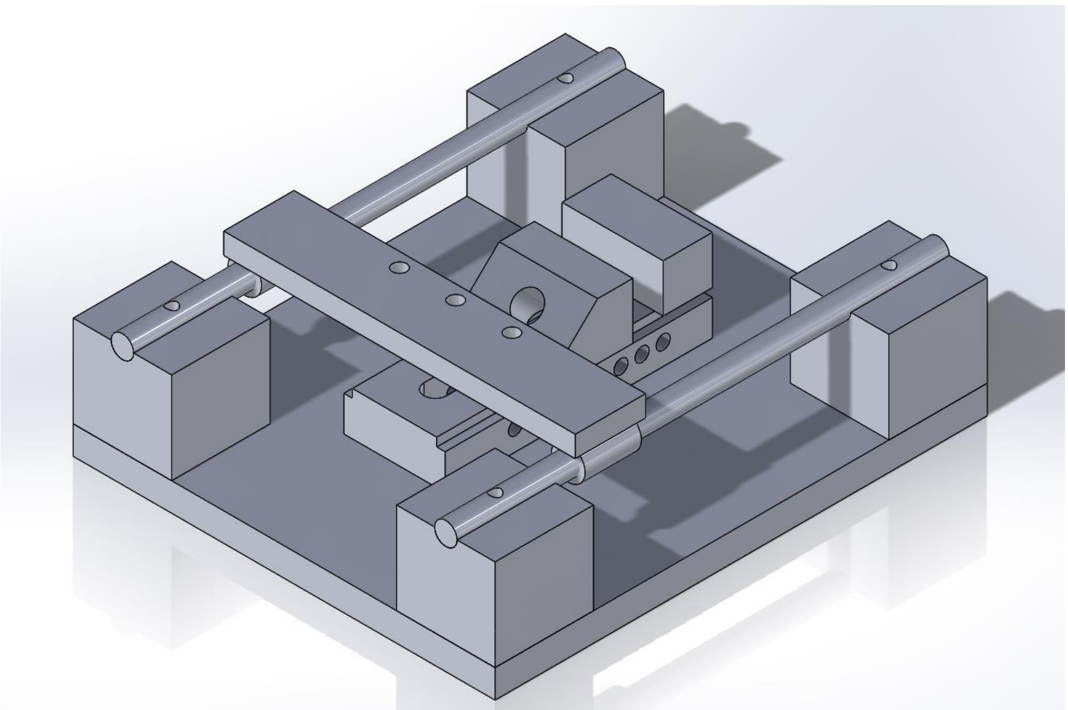

Figure 13: The initial design of the sliding microtome machine, which was not used, due to the inconsistencies when applying the force by hand which were noticed when testing with the handheld microtome. Designed using SolidWorks.

The sample holder, along with the blades were bought and used as received or with minor modifications. The pneumatic actuator was available from another project. All the other parts of the microtome such as the base and the blade holder were designed and produced for the microtome.

Before starting the design process on the microtome, there was a thorough search of different microtome machines available commercially and reading through patents used for them.
There is a vast number of different microtomes available on the market, ranging from simple handheld cutters, to devices with oscillating blades, or laser cutters. When designing the microtome, the original aims was that the device would be able to cut uniform sections at a precision of 10-40 µm. The thought was that the difficulties would lie in getting precise enough sections, and that the device would be stable and sturdy enough, especially considering the budget and time constraints. The plan was for the device to be as simple as possible, with few moving and technical parts, so that there would be less maintenance and would be easy to use. The goal was to do as much as possible in-house, using existing materials and parts, and through that save time and the budget.

4.2 Design Process

The design process was an ever-evolving cycle, and the environment, available materials and equipment all played a crucial part in the final result. The first design was a sliding microtome, using two cylindrical axels on both sides of the blade holder. The blade would slide over the sample holder which was connected to the axel with bushings. This idea was scrapped for a couple of reasons. The main reasons were that, with so many parts that would have to custom made it would be hard to achieve absolute stability and that as the force would be applied by hand, it would be challenging to apply an even amount of force, which is crucial when wanting to cut uniform thin sections.
To use a pneumatic actuator was by no means the first choice when designing the microtome.

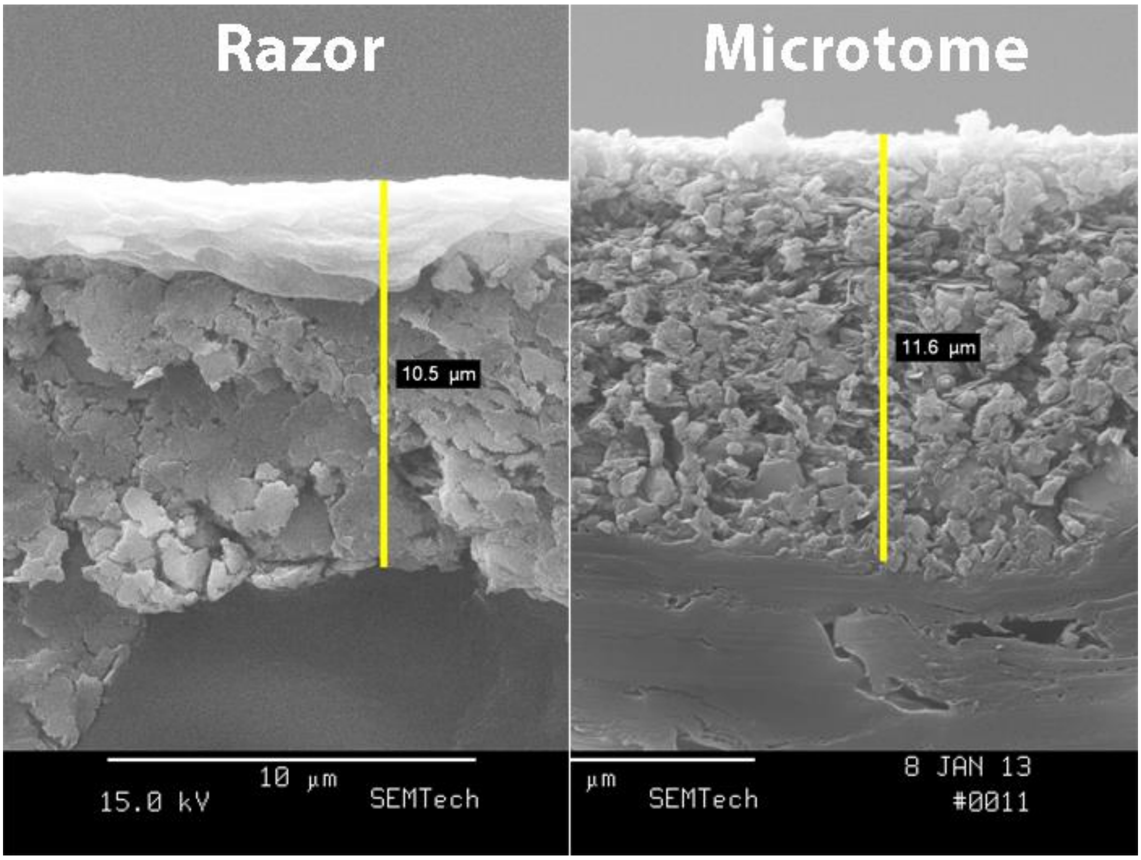

Figure 14: The difference of quality on a thin section, when cut with a handheld microtome with a razor blade (left) and with a Sorvall JB-4A microtome (right). The surface on the microtome cut is much more even, and less crumpled. (Picture taken from www.semtechsolutions.com)

4.3 Material Selection of Parts

4.3.1 Blade holder

The blade holder or sled is fastened to the pneumatic actuator and holds the blade that does the cutting on the microtome. The actuator pushes the blade holder towards the sample thus cutting the thin section.

The blade holder would have to endure a moderate amount of force, and the bottom part of the holder would slide against the sample holder. With these specifications in mind, Ertalon LFX was chosen for this microtome.

Ertalon LFX is an internally-lubricated cast Nylon 6, and it was developed for unlubricated highly loaded and slow-moving part applications. Ertalon LFX offers a reduced coefficient of friction compared to normal cast Nylon, which in turn increases its pressure-velocity capabilities and improved wear resistance. (Quadrant Engineering Plastics Products, n.d.)

4.3.2 Pneumatic Actuator

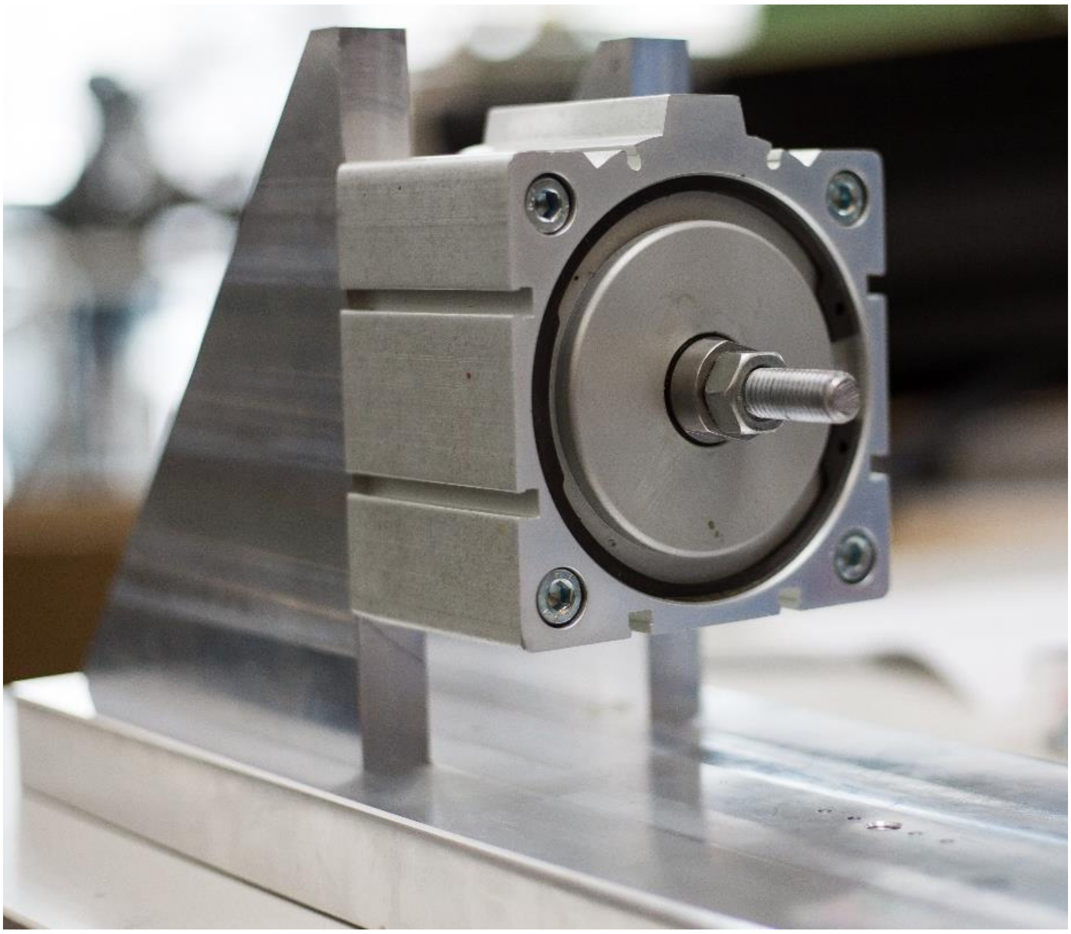

Figure 15: The Festo Pneumatic Actuator without blade holder, mounted on the microtome base. (Picture provided by Oscar Tulander, 2018)

After deciding that a hand cutting was not precise or consistent enough, another way to cut the thin-sections had to be found.

The pneumatic actuator, which was acquired from another project, was a Festo short-stroke cylinder (Festo ADVC-10-25-A-P). A pneumatic actuator or pneumatic drive uses compressed air to move the cylinder horizontally. The actutator is connected by hoses and valves to a source of compressed air. The blade holder would be mounted on the moving cylinder.

The actuator is a short-stroke cylinder and the datasheet states that this kind of cylinder is ideal for clamping tasks with short strokes. The actuator is very simple, can only move forward and backwards, and is controlled by a handle, which is positioned between the compressed air source and the actuator. It has a striking distance of 25mm and its maximum pressure is 10 bar. The actuator was equipped with valves that could regulate the movement speed of the blade holder.
The aluminum base for the microtome was made specifically with the pneumatic actuator in mind. The actuator is fastened on the pillars with four bolts.

4.3.3 Base and Pillars

The base and pillars were the last pieces designed for the microtome as, they entirely depended on the size and properties of the other parts of the microtome. The microtome already used some pretty heavy equipment as the steel sample holder, and the actuator. There also was a quite a bit of force in the system, so the base and pillars needed to be very precisely cut and remain incredibly stable as any wobble or instability could ruin the quality of the thin section.
The base was milled out of a cast aluminum plate.

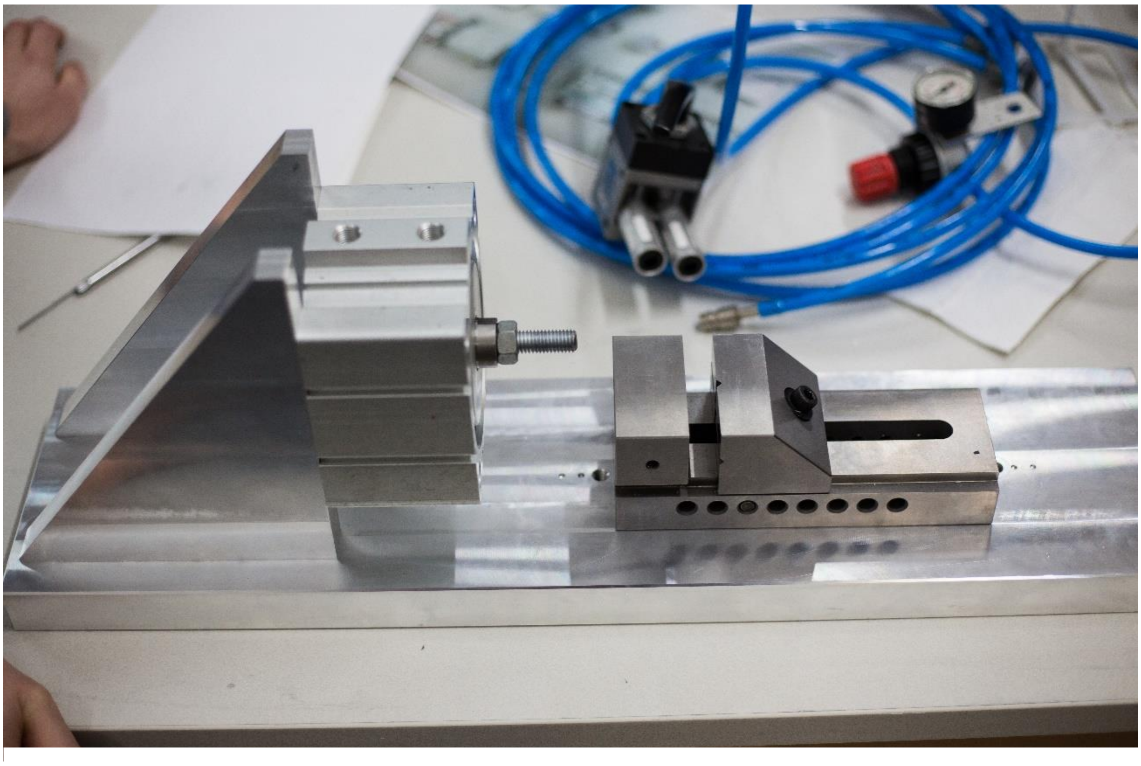

Figure 16: The base and pillars, both milled out of cast aluminum plate. They were designed using MasterCAM and then cut using a HAAS mill. (Picture provided by Oscar Tulander, 2018)

4.4 Fabrication or Acquisition of Parts

4.4.1 Blade and Blade Holder

There is much freedom when it comes to designing the blade holder as there is not any standard to adhere to. The blade holder was designed using SolidWorks after deciding on which blade to use. When designing the blade holder, the starting point was the pneumatic actuator and the blades as those two had already been chosen, and the holder would be the link between them. The design was evolving all the time and many different designs were made on SolidWorks before deciding on the final one.
After the design was finalized, the milling of two of the blade holders were outsourced to Isevat Oy[1], and a third final one was made in house using the HAAS mill.

The blade holder is easily changeable by design, as they all use the same blade and are mounted in the exact same way.
The blade holder is fastened directly to the actuator, by the threaded hole in the back side of the blade holder. The holder is fitted with three holes at the front, so that the blade can be fastened. As the blade holder acts as a sled, sliding on the sample holder, there was worry that where could be horizontal and vertical "wobble", causing inconsistent thin sections. The horizontal wobble was negated by cutting a recess on the bottom of the blade holder. By cutting the recess on the blade holder, the sides of the blade holder over-lap the sample holder. hus there can be no horizontal wobble. The recess on the bottom of the holders are only on the holders outsourced to Isevat Oy.

4.4.2 Aluminium Base Plate

The fabrication of the base for the microtome started with acquiring a sturdy aluminum plate (dimensions were 150 x 600 x 25 mm). The aluminum plate was machined using a HAAS mill. The part was designed on SolidWorks and the aim for the part was to be the base for all the other parts of the microtome.

[1] Although the initial stated aim was to fabricate all parts in Arcada, access to Arcada's HAAS mill was not possible at the time when the blade holder needed to be fabricated.

4.4.2.1 Pillars for the Base

The two pillars holding the pneumatic actuator were also milled out of a cast aluminum plate with the HAAS mill. The pillars were designed so that more fastening holes could be added to them, if the height of the pneumatic actuator needed to be adjusted.

4.5 Assembly of the Microtome

After the all the pieces of the microtome were fabricated or bought, the microtome had to be assembled. All the parts were precisely modeled using SolidWorks, so that their compatibility could be checked. All the positions of holes for the fastening bolts were also initially modeled in SolidWorks, so that it would be easy to check for problems before cutting into the parts. After the parts were modeled in SolidWorks, all the cutting routes were planned using MasterCam.

Figure 17: The assembled pneumatic microtome, without the hoses connected. The different parts in the microtome are (A) the valves, where the pneumatic hoses are connected. The valves allows for the cutting and retracting speed to be adjusted. (B) The Festo pneumatic drive, fastened on the pillar, with bolts. (C) The changeable Ertalon LFX blade holder. The blade holder in the picture is the one with a 45º cutting angle. (D) The Makita high-speed steel blade. (E) A sample, ready to be cut. The sample in the picture is UHMWPE. (F) The Xueling precision vise, used as the sample holder. (G) The sample holder is fastened to the base of the microtome using washers and a bolt. An identical solution is used on the other side of the vise. (H) The aluminium pillars, which are fastened on to the base of the microtome with bolts. The pneumatic drive is fastened on to the aluminium pillars. (I) the aluminium base of the microtome, on which the microtome is built.

5 EXPERIMENTAL TESTING OF PNEUMATIC MICROTOME WITH POLYMER SAMPLES

5.1 Samples Used

For testing the microtome, different samples with different manufacturing methods were acquired. The samples were extruded, 3D-printed, or injection molded. The samples could be of various sizes and shapes, but were constrained to a maximum thickness of 25 mm due to the striking distance of the actuator, however the optimal thickness of samples was approximately 20 mm.

The 3D-printed samples were made specifically to be used in the microtome. The samples were made out of PLA and were provided by Arcada.

To get the microtome to work properly, a decent number of different polymers with different material properties had to be tested. These materials were not used for analysis but to find the parameters needed to obtain thin sections with the microtome.

A large amount of different extruded samples was obtained from Etra Oy. The materials of these polymers were, for example, PTFE, HDPE and POM. The samples were cut to a suitable size with a band saw and then mounted on to the sample holder.

5.2 Experimental method

5.2.1 Sample Preparation

After the thin sections are cut from the sample, they may be ready to be placed on a microscope slide and be analyzed, but there are a few common problems that need to be fixed before analyzing.

Preparing a sample for microtomy is straightforward as the sample can be inserted to the holder as is, without any additional preparations, it the sample fits. There are however size constraints because of the striking distance of the pneumatic actuator, so the sample cannot be more than 25 mm thick. If the sample is too thick or rough, Scheirs (2000) states that while a hack-saw can be used to cut the sample into a more appropriate shape, a band-saw gives better results because it leaves less smearing on the face of the sample, due to the higher cutting speed of the band saw. Scheirs also noted that for polymers with low melting temperatures (for example low density polyethylene, LDPE), it is highly important not to overheat the sample during the cutting process. Thermosets and other very brittle or fiber reinforced polymers, are usually not suitable for microtomy, and polishing is the recommended way to obtain their thin sections.

Some samples may have odd or irregular shapes, or the material could be brittle or soft. For these samples Scheirs (2000) recommended that they be cemented to a substrate with a two-component adhesive, for example an epoxy or acrylic adhesive. Epoxy will also help if the sample is delicate, as the epoxy will stabilize the polymer.

The samples can be cleaned using an ultrasonic cleaner, but it is not mandatory.

5.2.2 Mounting Samples

The sample can be mounted into the sample holder in any direction. When mounting the sample, it is important that the free part of the sample (i.e. the part sticking out of the sample holder) does not exceed 2 mm, as it leads to more stress on the part and the blade. Depending on the polymers modulus, the specimen may bend and deform.

The sample is placed on to the holder and fastened so that the sample is still a bit loose. The height of the sample is regulated by placing a plate with the desired cutting thickness around the sample, and then the sample is pressed down. After the desired height is achieved, the sample is fastened more tightly. While the sample must be so tight that it will not move when a bit of pressure is applied, if it is fastened too tightly the sample may deform. Any minor movement will become greater as pressure is applied by the blade.

5.2.3 Microtomy

When the sample is adjusted correctly and fastened in the sample holder, the next step is to cut the thin section. For optimal results the cutting should occur in steps of 5 µm (i.e. cut 5 µm of the sample with each cut) (Scheirs, 2000).

Here it is important that the angle of the blade is positioned correctly, depending on the shape and material of the sample, as if the blade angle is too steep or shallow, the blade may dig in to the sample and break it, or if the cutting angle is too shallow, it may damage the surface of the specimen, as shown in Figure 5.

While the preferred thickness of thin sections is stated as 10-40 µm, it is advised to prepare sections from 100 µm down to 10 µm to see in which thickness the failure of the part can be best identified.

The cutting force can be adjusted from the compressed air source. The pneumatic actuator can cut with a pressure of up to 10 bars, but that is not necessary, and will most likely lead to poor and broken thin sections. The optimal cutting force, depending on the material in between 1.5-6 bars of pressure. When cutting with high pressure care should be taken as if the sample is hard, it may break and burst of the rest of the sample, leading to a broken sample.

The cutting and retracting speed can be adjusted with the two valves on top of the actuator. While the retracting speed has no difference for the thin section, the cutting speed affects the quality of the section.

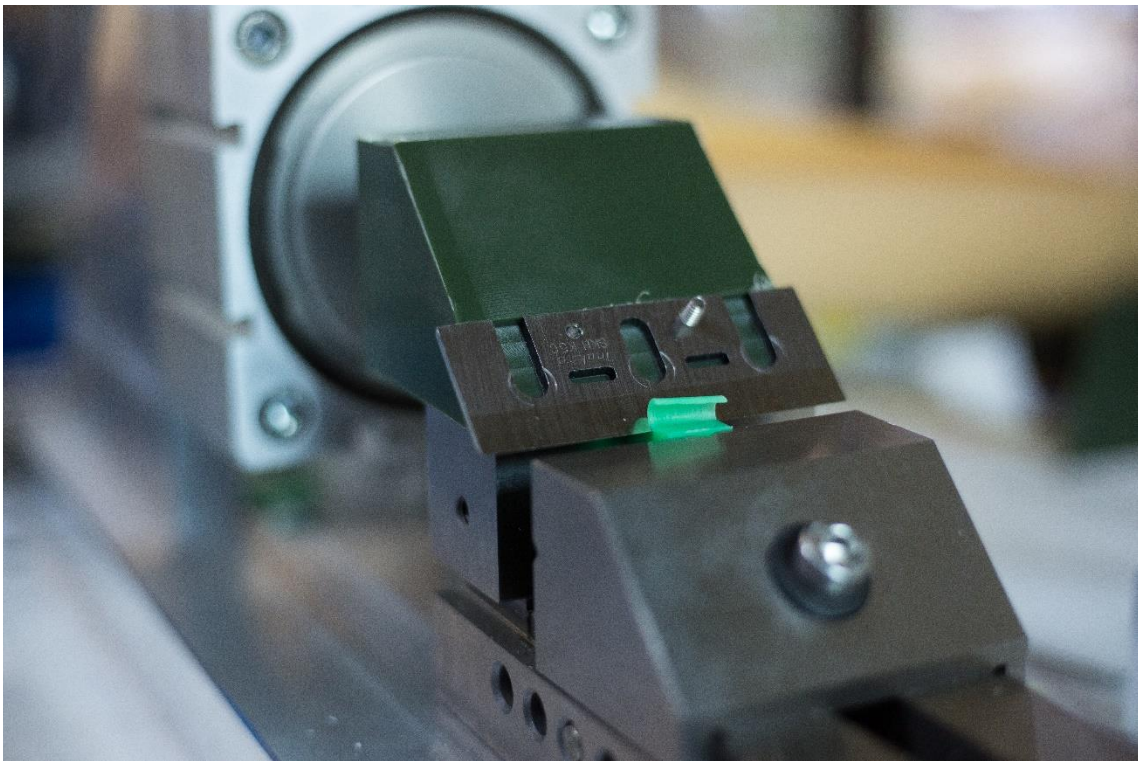

Figure 18: The microtome mid cut, with all cutting parameters adjusted. The sample being cut is a piece of UHDWPE. After the cut the thin section is ready for analysis. (Picture provided by Oscar Tulander, 2018)

5.2.4 Analytical Methods

5.2.4.1 Measuring the Thin-Section

Figure 19: A thin section being measured using a micrometer. The material of the smaple is PTFE. On some softer samples it may be good to keep the section under the micrometer for a while, for an accurate thickness.. (Picture provided by Oscar Tulander, 2018)

After a thin section has been cut, the thickness of it can be measured using a micrometer. The thin section should only be handled using tweezers, as not to contaminate the section. Before measuring the section, the micrometer should be calibrated to 0 µm. The needle the micrometer should be pulled up, and the section can be placed under the needle. If the thin section is hard to handle, it can be mounted on a microscope slide first, but the thickness of the slide and the glue used (Canada balsam, quick epoxy etc.) need to be measured also. It is important when measuring the thin section, that it is measured at multiple points. The thin section can be moved while under the micrometer, by using the tweezers. The thin section does not need to be totally uniform, but if the thickness differs too much on the thin section it will alter the profile of it when analyzed with a microscope.

5.2.5 Optical Microscopy

The thin-sections obtained by using a microtome are analyzed using an optical microscope. The microscope used to study the obtained thin section in this study is a Zeiss Axio Scope.A1. The microscope can be used in multiple different viewing modes, and different filters can also be used. The magnifications available are 5x, 10x, 20x, 40x, 50x and 100x

The microscope images can be viewed through ocular lenses, but there is also a camera mounted on top of it, with a monitor connected. The camera can be used to take pictures of the specimen, or to project the image on to the monitor. The different viewing modes on the microscope are:

- Dark Field (DF) (reflected light)
- Differential Interference Contrast (DIC) (reflected light)
- Bright Field (BF) (For reflected light and transmitted light)

The microscope can used reflected or transmitted light to illuminate the sample. Transmitted light is used for analysis of thin sections.

Optical microscopes are popular to use for polymer analysis due to their relatively low cost, because it is simple to prepare the sample for analysis and due to their ease of use.

The resolution of optical microscopy is between 0.2-1 μm (resolution is defined for an optical microscope as the shortest distance between two point that can be distinguished by the operator). The limit of the resolution is based on the wavelength of visible light.

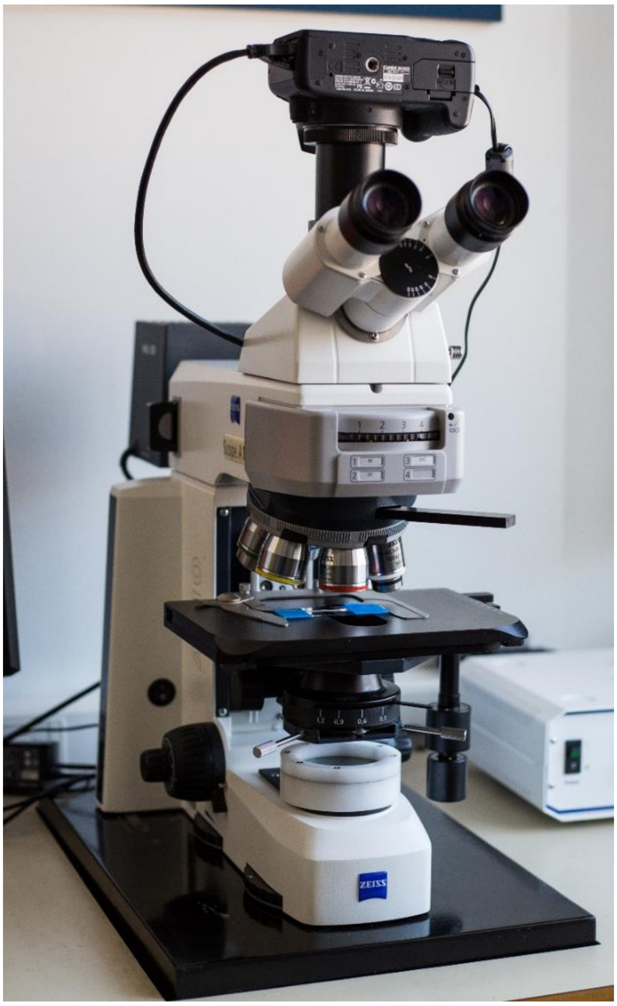

Figure 20: The Zeiss Axio Scope.A1 used to analyze the thin sections. (Picture provided by Oscar Tulander, 2018)

5.3 Identifying Defects and Structures with Optical Microscopy

The purpose of failure analysis of polymers, as well as other materials, is to find a cause of failure and through that a way to prevent it. Analysis of thin sections obtained with microtomy, are designed to test the materials properties, its processing history and service conditions.

5.4 Results and Analysis

5.4.1 Experimental results

All the samples were cut to an approximate width of 20 mm with a band saw prior to microtomy. All the samples were totally untreated prior to microtomy. While the cutting speed can be adjusted from the valves on top of the pneumatic actutator, all the samples used the same cutting speed. The height of the sample was regulated using the method described in chapter 5.2.2.

The section thickness was measured using the method described in chapter 5.4.2.1.

The sample width was controlled with a digital caliper.

Some samples such as 3D-Printed recycled PP were not tested as they would have required prior treatment as the form of the sample was not suitable for the sample holder.

Table 4: A table containing cutting parameters and results of various sample materials. "Pneumatic pressure" is the pressure used when the microtome was able to cut a section of the material. "Sample width" is the width of the cutting area of the sample. "Section thickness" is the thickness of the best thin section obtained, if one was obtained. "Observation" contains short observations made when cutting. "Suitable for hand-microtome" is if it was possible to cut a section of the material using the hand-microtome.

Sample material	Pneumatic Pressure	Sample Width	Section Thickness	Observations	Suitable for Hand-Microtome
UHMWPE	1,5 bar	19,95 mm	50 µm	Suitable for microtomy	Yes
PTFE	1,5 bar	20,05 mm	70 µm	Suitable for microtomy	Yes
3D-Printed PLA	2 bar	20,00 mm	N/A	Breaks, too brittle/hard	No, Sample breaks
PC	2 bar	21,15 mm	N/A	Breaks, too brittle/hard	Yes
PEHD 300	5 bar	20,05 mm	N/A	Breaks	No
PETP	5 bar	20,20 mm	N/A	Hard, breaks	No

5.4.2 Interpretation of the Results

To get a better understanding on what materials are suitable for microtomy, a wider array of samples should be tested.

Of the samples tested only UHMWPE and PTFE were suitable for microtomy without any treatments. The crystallinity of the samples seem to play a big role in the samples suitability for microtomy. UHMWPE and PTFE were the only semi-crystalline samples used in the testing (Sobieraj M, 2008) (Rae & Dattelbaum, 2004).

The very hard or brittle samples seem to be more suitable for polishing, as they broke from the pressure of the pneumatic actuator.

The testing could be improved by using a wider array of samples, especially more testing of semi-crystalline samples. The samples that were not suitable for microtomy could be tested again after treating them.

5.4.3 Problem Issues and Solutions

There were numerous issues, along with broken parts when constructing the microtome. The biggest problems arose from fitting all the parts. There cannot be any instabilities or wobbles anywhere on the microtome, as that would lead to non-uniform thin-sections. If the sample or any other part on the microtome is not fastened correctly or hard enough there is the possibility of breaking the sample or the blade.

If the sample holder is not fastened correctly to the base, and the sample is hard, the sample holder can rise from the base. If that happens there is the possibility of the blade colliding with the sample holder, leading to a shattered blade

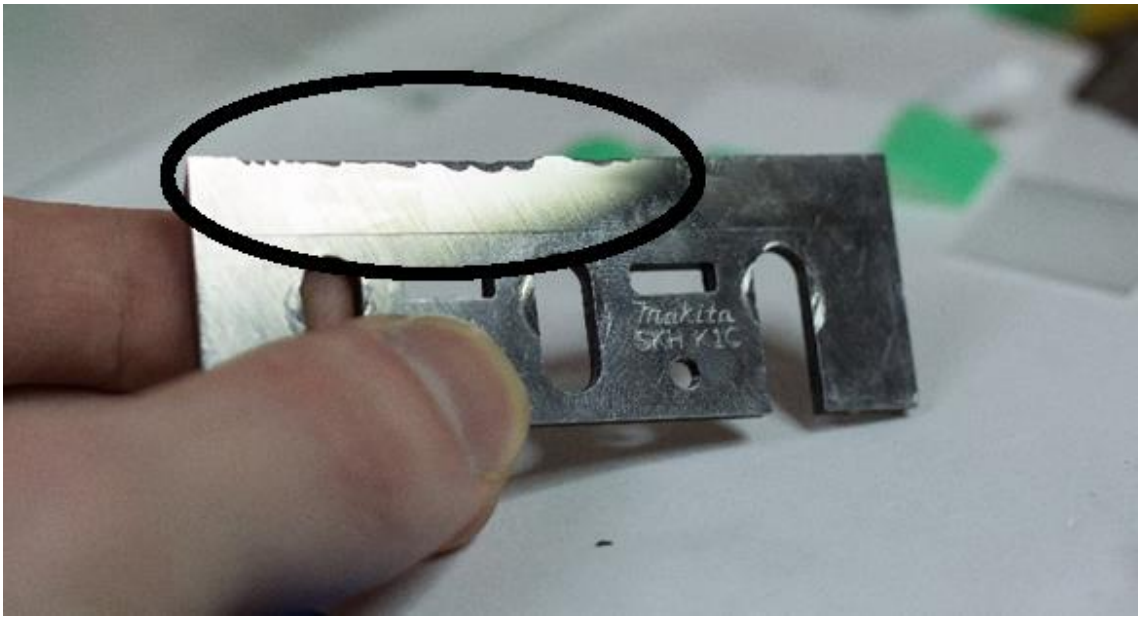

Figure 21:The blade after it shattered. The blade shattered as it hit the sample holder, because the blade dug into the sample. This was fixed by adjusting cutting parameter and fastening the sample holder harder. (Picture provided by Oscar Tulander, 2018, Edited using GIMP)

Another issue was that when the cutting is in progress, the blade can dig into the sample. When that happens, the bottom of the blade holder collides with the sample holder which can damage the blade holder. This can be fixed by adjusting the cutting speed, or the cutting force.

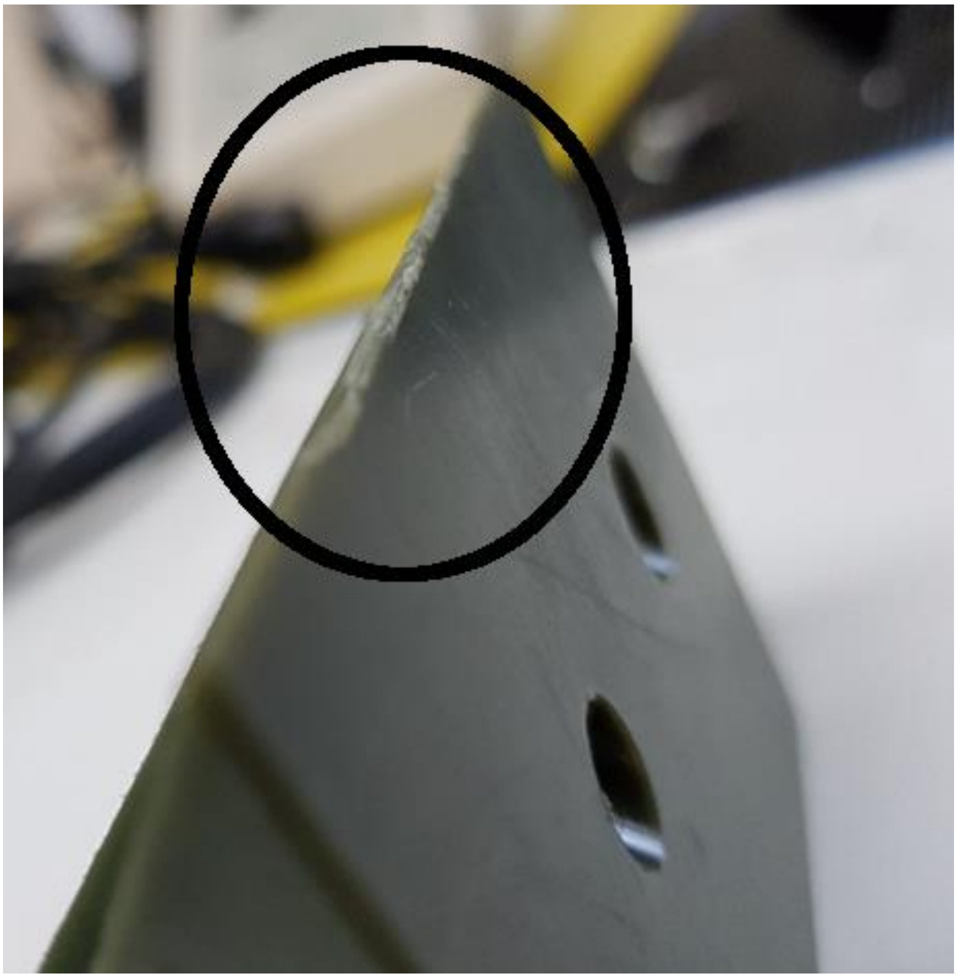

Figure 22: The blade holder wears down if not fastened correctly as it collides with the sample holder. (Picture edited on GIMP)

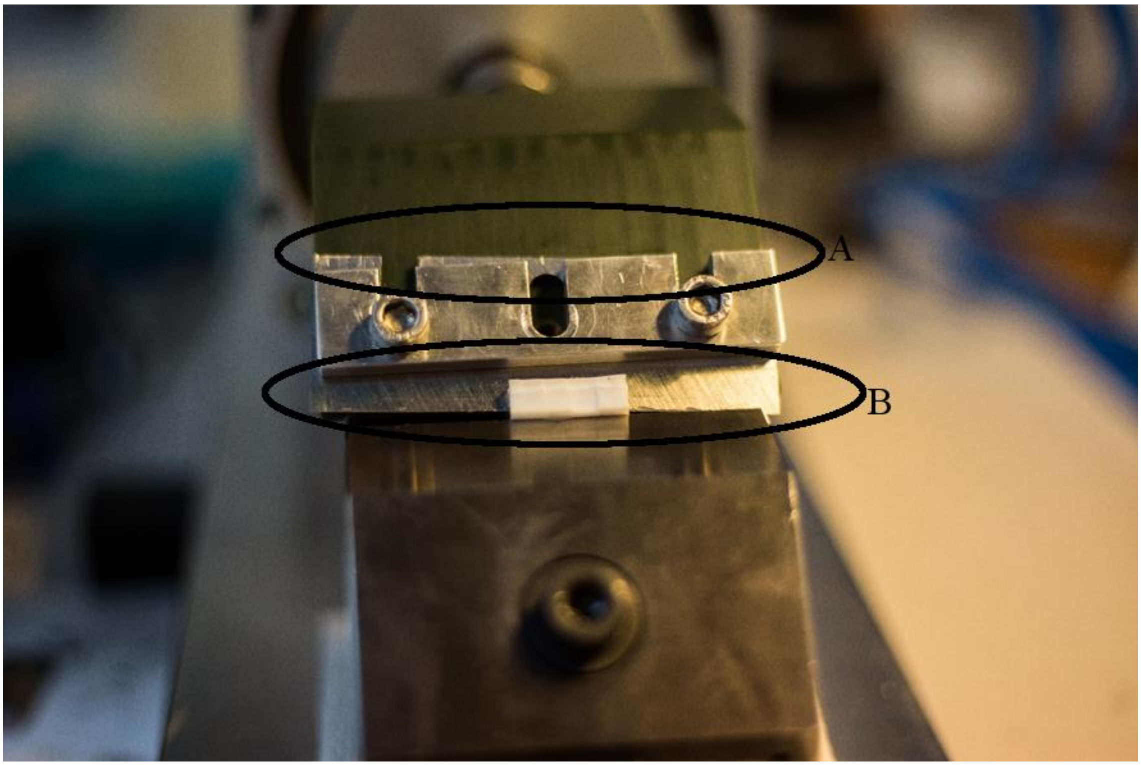

Figure 23: The blade is unevenly fastened, which means that the blade will not cut a uniform thin section (A). As the blade is not aligned with the sample holder, it will cut into the vise, which will damage the vise and shatter the blade (Photo provided by Oscar Tulander, 2018.Edited using GIMP)

Some issues may also arise if the wrong cutting force or speed is used. If the cutting force is too low, the blade will not cut through the sample, and only leave some marks on the surface of it. If the blade "pushes" on the sample, it may cause stress inside the sample, leading to poor and curled up thin sections.

On some hard and brittle samples, such as polycarbonate the sample may easily shatter if the cutting speed or force were not adjusted correctly.

6 COMPARISON WITH OTHER METHODS

6.1 Comparison to Polishing

Obtaining thin section by microtoming is the simplest way, but there are various different methods to obtain them.

Generally microtoming produces good samples very fast, with a sample being produced in about 10-20 minutes. A microtome machine can however be very expensive. Polishing produces higher quality samples but they require much more work. First embedding the sample in epoxy, then polishing and mounting it. To produce a sample by polishing can take up to two days, with a few hours of work per samples. Polishing also requires more equipment like the polishing machine, and various different grains of sand paper, glue and an embedding resin.

When choosing the way to obtain thin sections for microscopy, a few different things should be taken into account. Viewing angle required, preparation time and the nature of the component from where the thin section will be made are examples of things that should be taken into account when choosing. One big advantage of polishing is that it allows fractured or broken samples to be observed internally and at angles which are not possible by microtomy. Additionally, thin sections obtained by a microtome can be distorted or stretched which would not happen with polishing. Microtoming however remains the preferred method of preparing samples.

Certain polymers cannot be microtomed due to how brittle they are, examples of these polymers are thermosets or fiber reinforced (glass fiber or carbon fiber) plastics and composites. Polishing is the preferred method to obtain thin sections of these materials. While it is possible to produce thin sections with a microtome of composites, it is very hard to produce thin sections of high quality as fibers are generally torn during the microtome process.

3D-printed samples of polylactic acid (PLA) were tested with both methods. The samples provided for microtoming had a layer height of 0.2 mm. While the microtome was able to cut the PLA samples with no issues, it was not possible to obtain thin section suitable for microscopy. The sections shattered due to how hard and brittle the material was. There were, however no problems when polishing the same material.

6.2 Microtomy Used Together with Polishing

Microtoming could also be used as a complimentary method for polishing to obtain thin sections, and vice versa. Using a microtome to cut a thin section to be relatively thin, would cut down on the polishing time. The thin section would still need to be embedded, so it would still be time consuming.

Polishing could be used to plane or to shape the sample into a better shape for sectioning, without putting the sample through too much stress or altering its morphology.

6.3 Selecting Microtomy or Polishing – Or using a combination of the two methods

While polishing can be used on almost any material, some materials can be too tough. For example, the UHMWPE (ultra-high molecular weight polyethylene) is very hard to polish, partly due to the extremely long polyethylene chains (Kurtz, 2001). Some materials cannot be embedded in epoxy or other resins such as polytetrafluoroethylene (also known as Teflon), and thus cannot be polished. Both of these materials are suitable for microtoming. The strength of microtoming lies in its speed and ease of cutting thin sections.

The microtome can be used as a complementary method with polishing, as it can cut down on time needed for polishing by cutting an already thin section and then finishing it of by polishing.

3D-Printed materials were very difficult to microtome because of the layered nature of it. However, there were not any problems when polishing 3D-Printed materials. (The only 3D-printed materials tested were PLA).

While microtoming is the most used and fastest way of obtaining thin sections, polishing are the preferred methods to produce high quality composite samples.

There are arguments for both methods if a decision has to be made on which method to use. A simple microtome would be more affordable than all the equipment needed for the polishing method, and it would be most simple method if quick results are needed. Polishing however, while much more time-consuming, works on a bigger amount of samples, such as fractured specimens.

7 CONCLUSIONS

7.1 Summary of Work

There are many steps and much work to be done, when it comes designing any product, and perhaps even more so when it comes to designing lab equipment.

Before even starting the design process, the literature regarding how microtomes are used today, their applications and the various kinds of different microtomes had to be read. Thin sections are used in failure-, material- and composition analysis and are mainly analyzed using an optical microscope. Transmitted electron microscopy, scanning probe microscopy and scanning electron microscopy are also used to some degree. There are several kinds of microtomes, from simple handheld ones, to complicated stationary ones with oscillating blades.

The design process began with the simplest possible microtome. A handheld microtome made of POM equipped with a high-speed steel blade. The sample holder for the microtome was a precision steel vise. After experimenting with the handheld microtome, the conclusion was that while that there were no problems with the blade, the handheld microtome along with the unfastened sample holder led to inconsistent thin-sections.

The design then evolved to a pneumatic microtome. The pneumatic microtome used the same blade and sample holder, but the pneumatic drive moved the blade to make the cutting maneuver. The blade was mounted on a Nylon blade holder designed especially for the microtome. The wobbling and inconsistency issues with the sample holder were solved by fastening all the parts of the microtome on a custom made aluminium base.

The pneumatic microtome fabricated in this study was tested using multiple different injection moulded, 3D-printed and extruded samples. The microtomy process consisted of just a few steps. The first step was preparing samples by cutting and mounting them in the sample holder. When mounting samples, they had to be secure and free of “play” as any movement lead to inconsistent and jagged samples. When the pneumatic drive was connected to the compressed air source the microtome was ready to be used. The cutting force could be adjusted at the source of compressed air and the cutting speed adjusted by the valves on the pneumatic drive. After the thin sections were cut, they were finally mounted on microscope slides ready for analysis.

Microtomy is the fastest and most common way of producing thin sections for optical analysis, but another common and popular method is polishing. The polishing method produces higher quality samples, but the process takes much longer. The polishing process involves embedding the sample into a resin, and then polishing it down to 10-100 µm, before it can be analyzed.

7.2 Achievement of Goals

The original aim of the thesis was to generate a method to produce thin sections, which could be used as a part of Arcada's failure analysis capabilities which were a bit lacking. This thesis would concentrate on making the thin sections by microtomy, and another parallel thesis would produce thin sections by polishing. A pneumatic microtome capable of cutting consistent sections of various different materials was fabricated, and while the method of regulating the sample height needs improvement the goal was achieved.

7.3 Developing the Microtome Further

While both fabricated microtomes are capable of producing thin sections, both of them could be developed further.

The handheld microtome could be used as a complementary method with the pneumatic microtome.

The biggest remaining issue with the pneumatic microtome is regulating the height of the sample and the stability of the sample holder. The stability of the sample holder could be improved by making a better method of fastening the vise. Currently the vise is fastened by a large washer tightened by a bolt, on both sides of the sample holder. The fact that the holder is fastened by two different bolts, always leads to a chance of unevenness. A plan was made for the fasteners, but due to time constraints, they were never finished.

There were different ideas for a method to regulate the sample height for the microtome. One idea was to make a "bridge" which could be used to push the sample to a desired height. Another method could be to install a platform under the sample, which would be controlled by a micrometer screw.

8 SWEDISH SUMMARY

8.1 Introduktion

Syftet med detta examensarbete är att producera en metod för yrkeshögskolan Arcada att skaffa tunna tvärsnnitt av fromsprutade samt 3D-printade delar. Ett tunt tvärsnitt är en 10-40 µm tjock bit av ett material. Tvärsnittet används i fel, material samt komposition analys. Ett optiskt mikroskåp använnds föratt analysera tunna tvärsnitten.

Mikrotomimaskinen hittades på år 1865 av Schweiziska forskaren 1865. His beskrev hans uppfinning som ”En mekanisk maskin som används för att skära tunna vävnadssnitt för mikroskopisk undersökning”.
Medan det finns några olika metoder föratt skapa tunna tvärsnitt, så koncentrerar detta examensarbete på att skapa dem med en mikrotom maskin. En mikrotom maskin skär med en kniv en tunn sektion av material från en prov bit. Medan mikrotomer också används för att skära tvärsnitt att biologiska och geologiska prov bitar, så används den producerade mikrotomen bara för olika polymer material.

Mikrotom prossessen är relativt simpel. En provbit som kan vara av olika storlekar, blir fastsatt i provbits hållaren. Sedan skärs tvärsnittet loss med mikrotom kniven, som kan vara av olika material, men oftast av glas eller stål. Efter att tunna tvärsnittet är skuret, är den klar att läggas på ett mikroskop glas och sedan kan den analyseras med ett mikroskop.

För detta examensarbete, producerades två olika mikrotomer. En simpel handmanöverad, som består av ett POM handtag, och ett höghastighets stålblad. Andra mikrotomen som produserades använde samma stålblad, men skärande styrdes av en pneumatisk motor.

I denna examensarbete, jämförs mikrotomi också mot polering, som är en annan metod att producera tunna tvärsnitt.

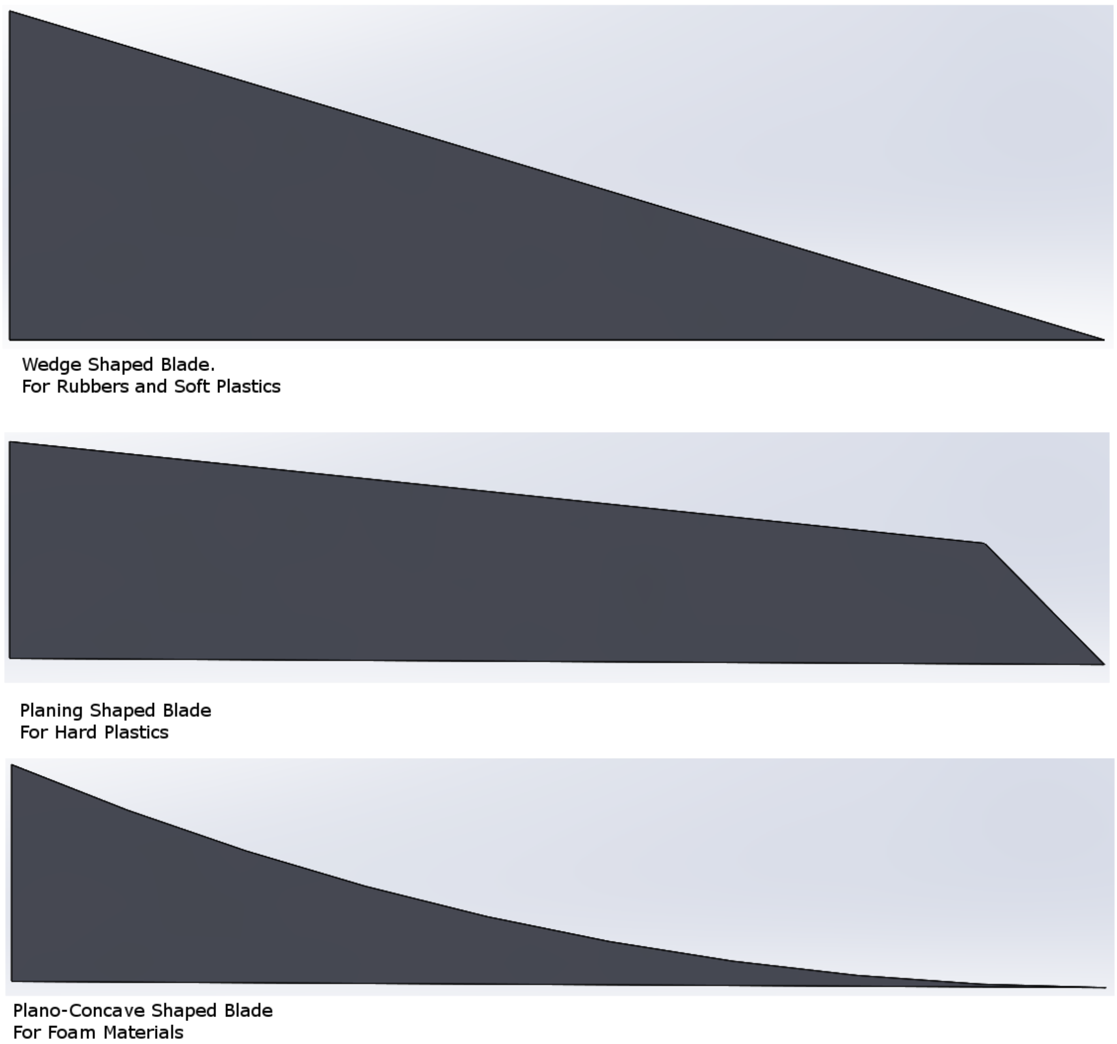

Figure 24: Olika typer av stål knivar som används i mikrotomi.

8.2 Mikrotomi Prosessen

Mikrotomi prosessen är relativt enkel. Urvalet av provbitar som kan användas inom mikrotomi är enorm, eftersom nästan vilken som helst polymer funkar. Med värmehärdiga eller hårda o spröda material kan de uppstå problem.

Efter att provbiten är vald, och fäst i provhållaren är den klar att bli skuren. Vissa material kan man inte rakt skära. T.ex. ostabila, för hårda, eller för mjuka material måste man bearbeta före de skärs. Ostabila material kan man också innesluta i epoxi, eller en annan liknande harts.

Som blad till mikrotomen kan det användas ett stort urval olika knivar. Glasknivar används ofta till ultramikrotomi, som betyder att tvärsnitten skall vara 0.1-10 µm tjocka.

Till vanlig polymer mikrotomi används oftast hård-stål knivar. Skärnings vinkel beror på provbitens material, men allmänna regeln är att är att vinkeln skall vara mellan 15º och 45º. Oftast desto högre vinkel, desto hårdare material. Också formen av kniven man använder beror på materialet. Den mest använda knivtypen är ett hyvel format blad.
När provbiten sitter ordentligt i provbitshållaren, är den klar att bli skuren. På vissa kommersiella mikrotomer kan man justera tjockleken av tvärsnittet man vill ha. Tjockleken bör vara mellan 10-40 µm. Om den är för tunn eller tjock kan det hända att all information man söker inte är tillgänglig.
Efter tunna tvärsnittet är skuret, skall den placeras på ett mikroskopglas med pincetter. När snittet placeras på mikroskop glaset, måste den hållas helt platt. Om den stiger eller rullar ihop, ser man inte all information med mikroskopet.
Att tunnatvärsnittet rullar ihop är det mest allmänna problemet med den här processen. Tvärsnittet rullar ihop på grund av inre stress som redan fanns i provbiten.
Det finns olika sätt att räta ut tvärsnittet. Snittet kan värmas upp i vatten, eller rullas upp med pincetter och sen läggas under en tyngd.
Om det inte hjälper, kan snittet limmas på mikroskopglaset med kanada balsam eller epoxi lim.
Snittet är sedan färdig att analyseras. Analysen sker med mikroskopet, och tunna tvärsnitt används för att hitta information om produktens historia, att hitta typ fel i mass producerade produkter, att hitta information om produktens gjutnings historia som t.ex flödemönster.

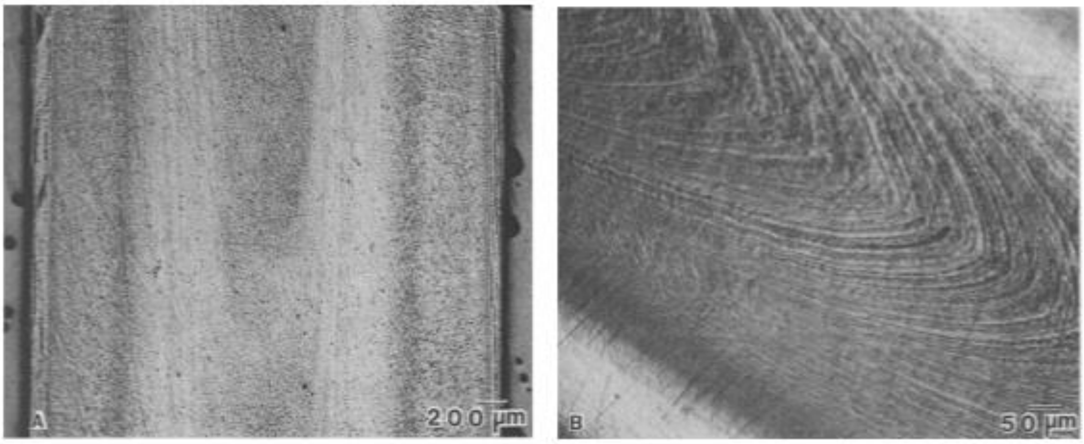

Figure 25: Flöde mönster på tvärsnitt av extruderade material. Upplyses av reflekterat ljus (vänster) och DIC (höger)

8.3 Tillverkning och Testande av Mikrotomerna

Två olika sorters mikrotomer producerades för detta project. En simpel hand-opererad mikrotom, och en stationär mikrotom som använde en pneumatisk motor för att skära tunna tvärsnitt från provbiten.
Första mikrotomen som gjordes för projektet, var handopererad och använde ett höghastighetsstål blad. Handtaget för mikrotomen gjordes av polyoximetylen (POM). Mikrotomen designades på SolidWorks. Efter att designen var klar, machinerades en POM bit till mikrotomed med en HAAS fräs. Hand-mikrotomen har två hål på fram delen, vart snabb stål bladet kan bli fäst.
Till provbitshållare valdes ett Xueling precision skruvstäd, gjord av 20 CrMnTi stål. Det behövdes en precisions skruvstäd eftersom den fungerade som stöd för skärande.

Hand-mikrotomen testandes på olika slags polymer prov. Provbitarna var av olika storlekar och former. Det uppstod inga problem med skärande av provbitarna, men när tvärsnitten mättes märktes de att de oftast var ojämna och hackiga. Problemet var att eftersom hand-mikrotomen opereras per hand är det otroligt svårt skära jämna tvärsnitt. Det uppstod också problem för att provbitshållaren inte var fastsatt, som tillät den att röra på sig. Ett problem var också att det inte fanns en tillräckligt precis metod för att reglera höjden av provbiten.

Figure 26: POM handtaget för hand-opererade mikrotomen.

Efter observationerna som gjordes angående hand-mikrotomen, bestämdes de att en stationär mikrotom skulle produceras. Målet med stationära mikrotomen var att den skulle fixa problemen som uppstod med hand-mikrotomen.

Det fanns inga problem med snabb-stål bladet, eller med provbitshållaren, så de skulle används till stationära mikrotomen, men allt annat som behövdes måste produceras eller skaffas.

En pneumatisk motor anskaffades från ett annat projekt. Bladet skulle monteras på pneumatiska motorn, vilket skulle avlägsna problemet med att skära för hand. Pneumatiska motorn använder tryck luft för att röra en stål cylinder horisontalt. Cylindern kan röra sig 25 mm.

Eftersom bladet inte kunde bli rakt monterat på cylindern, måste en bladhållare produceras. Bladhållaren producerades av Ertalon LFX som är ett självsmörjande nylon-6 material. Designen av bladhållaren planerades på SolidWorks, och maskinerades sedan men en HAAS fräs. Tre olika bladhållare producerades, med olika skärnings vinklar.

Bottnet för mikrotomen är verktygs aluminium, som maskinerades till rätt storlek, för mikrotomen men en HAAS fräs. Aluminium pelare maskinerades också. Pelarna fastsattes i bottnet, och pneumatiska motorn fastsattes i pelarna.

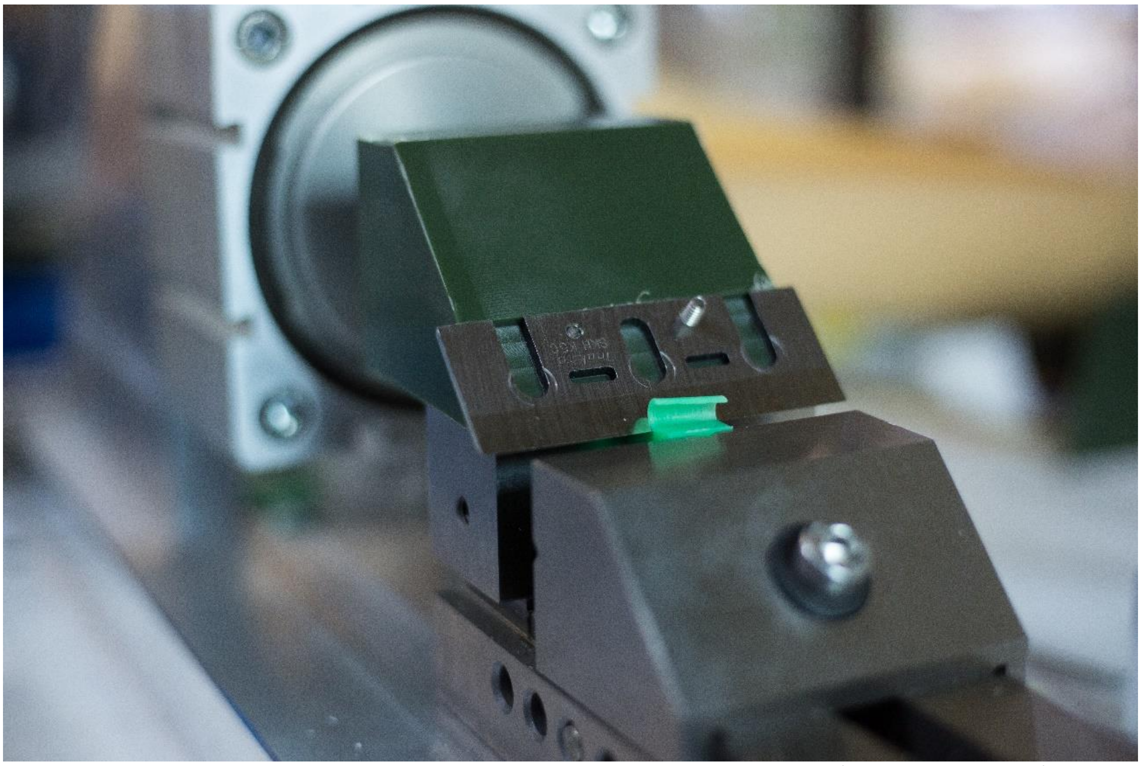

Figure 27: Pneumatiska microtomen, mitt i skärnings processen. Bladet är monterat på Ertalon LFX bladhållaren.

Pneumatiska mikrotomen testades med samma metoder som hand-opererade mikrotomen. Pneumatiska mikrotomen klarar av att skära enhetliga tvärsnitt av ett stort sortiment olika material. Skärnings hastigheten samt kraften är justerbar, vilket tillåter ett stort urval av provbitar att bli skurna.

Enda problemet som uppstår är justeringen av provbits höjd, vilket gör det svårt att få tunna-tvärsnitt inom 10-40 µm räckvidd.

Figure 28: Mikrotomen med alla delar monterade. (A) ventilerna var luft slangarna blir fastsätta. (B) pneumatiska motorn. (C) Ertalon LFX bladhållaren, med 45º skärnings vinkel. (D) Makita snabb-stål bladet. (E) Provbit monterad i provbitshållaren. (F) Xueling provbitshållare. (G) fastsättnings metoden för provbitshållaren. (H) Aluminium pelare, fastsatta I aluminium botnet. Pneumatiska motorn är fastsatt I pelarna. (I) Aluminium plattan, som fungerar som botten för hela mikrotomen.

8.4 Jämförelse Med Polering

Polering är en annan metod för att producera tunna-tvärsnitt av polymera provbitar. Medan mikrotomi producerar en provbit på 10-20 minuter kan polering ta 12-24 timmar.

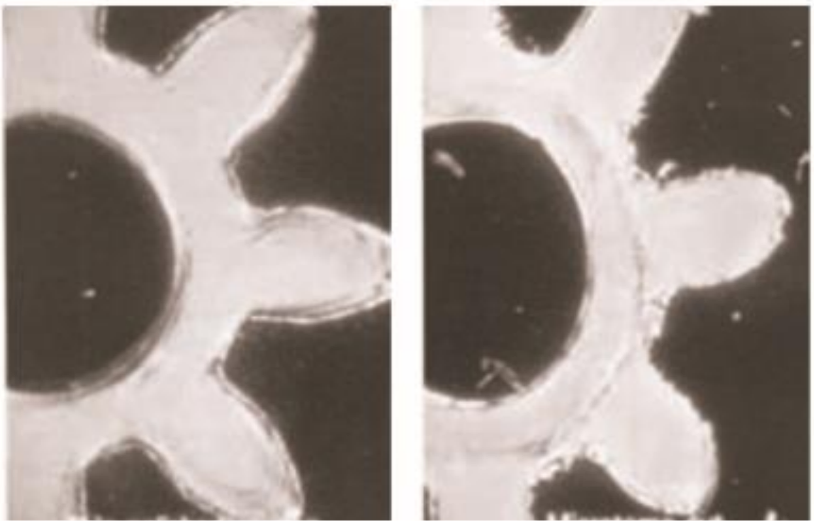

Figure 29: Ett polerat tvärsnitt (vänster) och ett mikrom tvärsnitt (höger).

När en provbit poleras, skall den först inneslutas I epoxi eller nån annan harts. Att hartsen härdas tar oftast manga timmar. När provbiten är innesluten, och hartsen härdad, skall provbiten poleras med en polerings maskin och sandpapper.

Poleringen sker i två steg. Först poleras ena sidan av provbiten, som sedan limmas fast i ett mikroskopglas. Efter att limmet har härdas mellan provbiten och mikroskopglaset, poleras också andra sidan av provbiten, ända tills provbiten är 10-100 μm tunn.

Medan polerings processen kräver mycket mera jobb och tid, anger den bättre resultat. Som Figur 6 visar blir tvärsnittet mindre deformerad och håller sin form bättre. Polerings processen tillåter också brytna provbitat att bli analyserade.

APPENDIX

8.4.1 Bill of Materials

Part	Part Name	Price	Quantity	Bought from
Blade	Makita SKH K1C planer blade	9,90€	2 pcs	ETRA OY
Base Plate	Aluminium Plate	150€	1 pcs	Uddeholm
Pneumatic Actuator (+hoses)	Festo Pneumatic Actuator (and hoses)	N/A	1 pcs	Festo
Blade Holder	Ertalon LFX	700 €	$1m^2$	ETRA OY
Sample Holder	Koneruuvipuris-tin VB 30	194,53€	1 pcs	Kauppa Osake Yhtiö

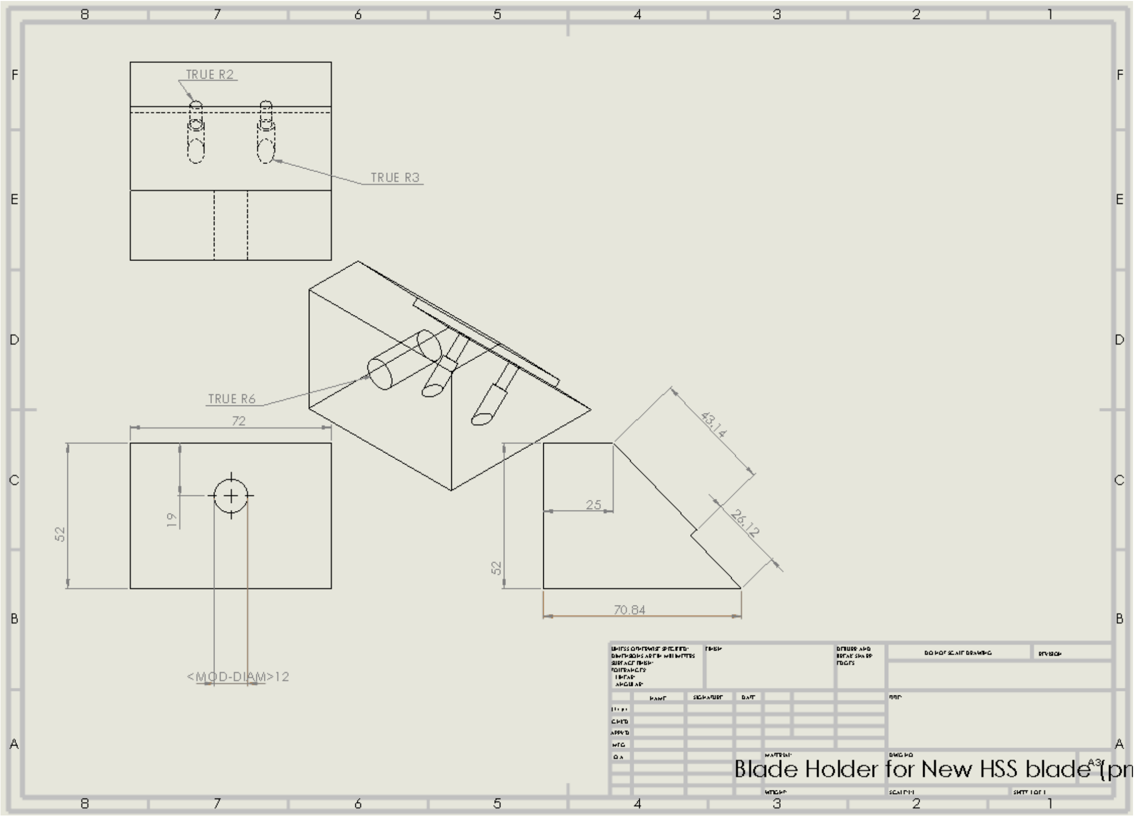

Figure 30: Engineering drawing for the blade holder (Drawing made on SolidWorks)

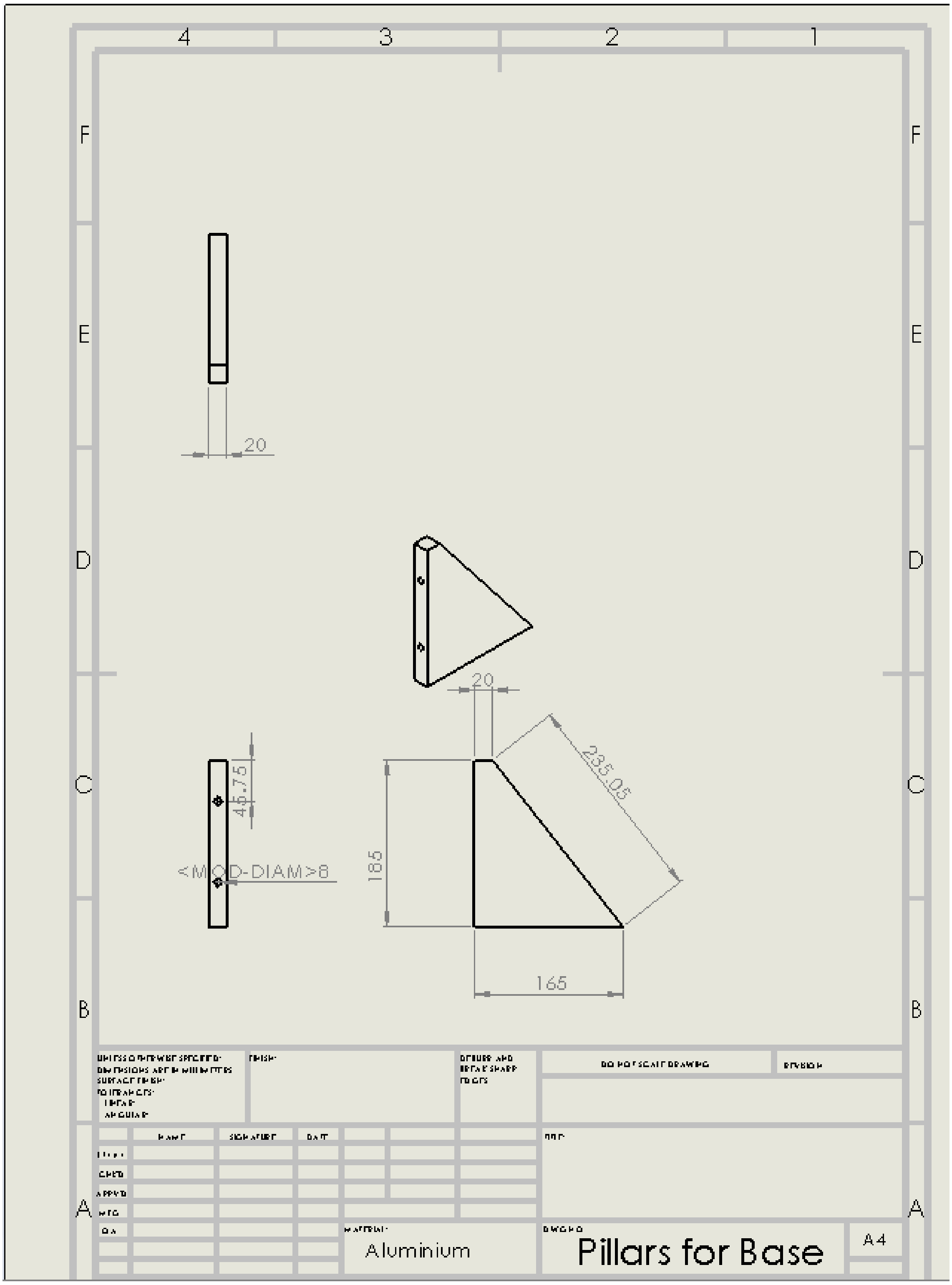

Figure 31: Engineering drawing for the aluminum pillars. (Drawing made on SolidWorks)

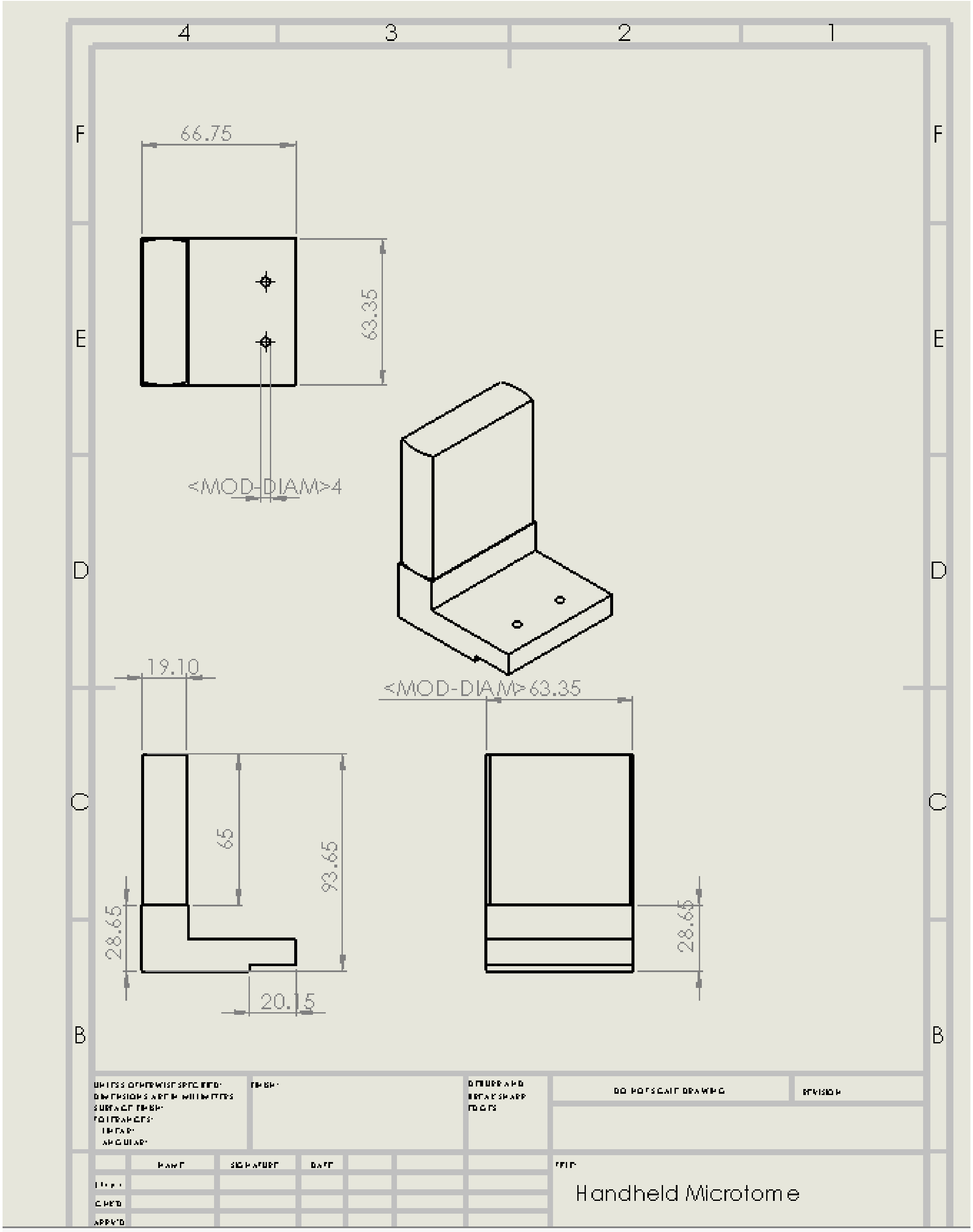

Figure 32: Engineering drawing for the POM handle for the handheld microtome. (Drawing made on SolidWorks)

BIBLIOGRAPHY

Brostow, W. & Corneliussen, R. D., 1986. *Failure of Plastics.* s.l.:Hanser Garden Publication.

Böhme, E., 1990. *Failure Analysis Using Microscopic Techniques.* Geneva: DuPont Engineering Polymers.

Erzin, M., 2013. *Plastics Failure Guide: Cause and Preventation.* 2nd Edition ed. Cincinnati: Hanser Publications.

Ibeh, C. C., 2011. *Thermoplastic Materials: Properties, Manufacturing Methods, and Applications.* 1st ed. s.l.:CRC Press.

Kurtz, S. M., 2001. *The UHMWPE Handbook: Ultra-High Molecular Weight Polyethylene in Total Joint Replacement.* San Diego: Elsevier Academic Press.

Otai Steel, n.d. *20CrMnTi Carburizing bearing Structural steel round bar.* [Online] Available at: http://www.otaisteel.com/products/bearing-steel-and-gear-steel/20crmnti-carburizing-structural-steel-bearing-steel-round-bar-20crmnti-steel/

Quadrant Engineering Plastics Products, n.d. *Ertalon LFX,* s.l.: s.n.

Rae, P. & Dattelbaum, D., 2004. *The properties of poly(tetrafluoroethylene) (PTFE) in compression,* s.l.: Elsevier.

Readon, A. C., 2010. *Metallurgy for the Non-Metallurgist.* s.l.:A S M International.

Rosato, D. & Rosato, M. G., 2000. *Injection Molding Handbook.* s.l.:Springer US.

Sawyer, L. C., Grubb, D. T. & Meyers, G. F., 2008. *Polymer Microscopy.* 3rd ed. s.l.:Springer.

Scheirs, J., 2000. *Compositional and Failure Analysis of Polymers: A Practical Approach.* s.l.:John Wiley & Sons .

Sobieraj M, R. C., 2008. Ultra High Molecular Weight Polyethylene: Mechanics, Morphology, and Clinical Behavior. *Journal of the mechanical behavior of biomedical materials,* 2(5), pp. 433-443.

The Editors of Encyclopaedia Britannica, 2012. *Wilhelm His.* [Online] Available at: https://www.britannica.com/biography/Wilhelm-His-Swiss-anatomist#ref282613

Wagner, J., 2012. *Handbook of Troubleshooting Plastics Process: A Practical Guide.* Somerset: Wiley.