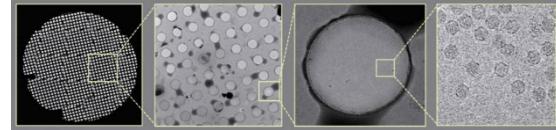
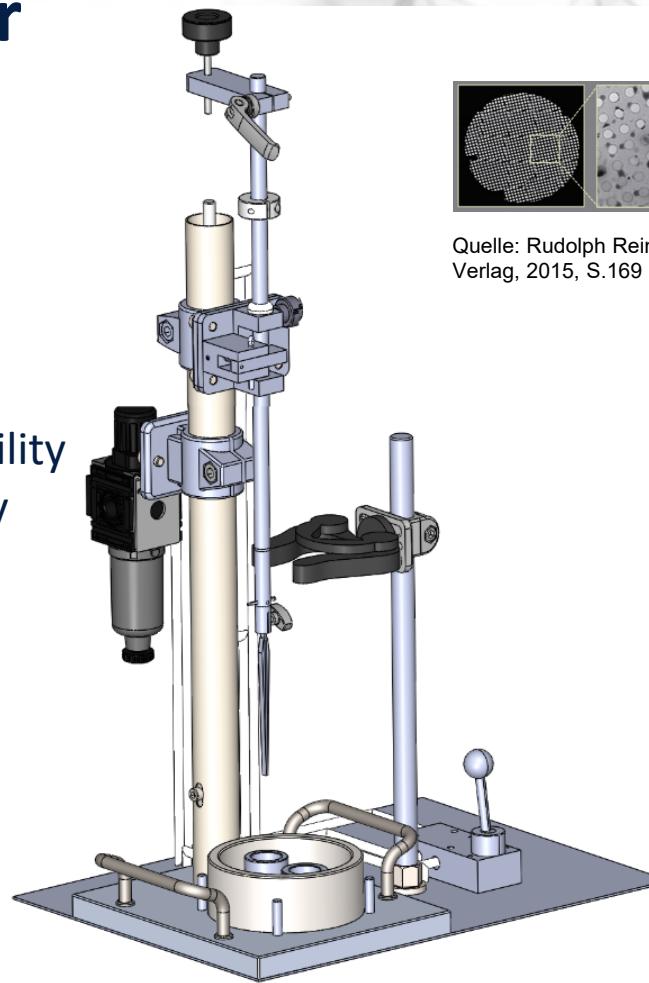


PEP2020-Plunge Freezer

Pit Silas Stücher, Silas Fischer,
Sali Luta, Hendrik Schneider

 **LMN SIEGEN** Micro- and Nanoanalytics Facility
University of Siegen, Germany

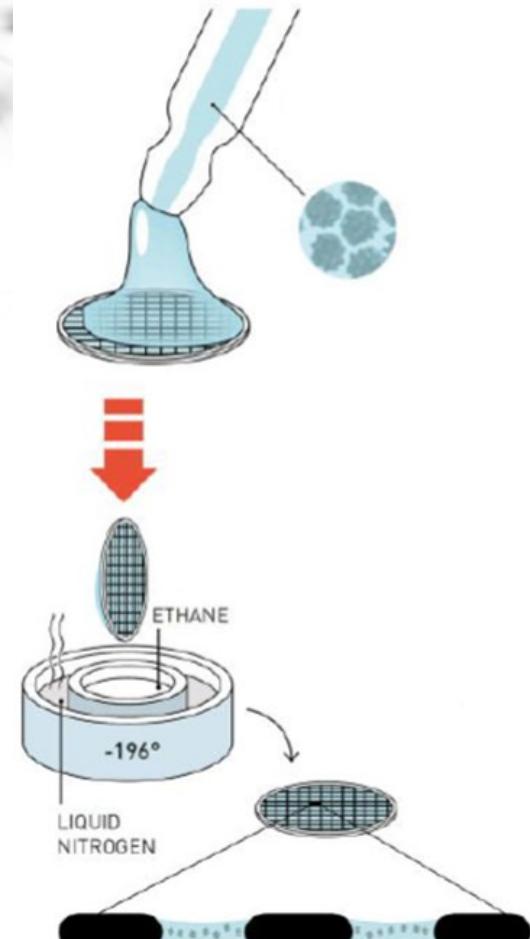


Quelle: Rudolph Reimer: Kryotechniken. Springer Verlag, 2015, S.169



Introduktion

To be able to investigate the material and (bio-)medical research samples, which are available as suspension, dispersion, biofluid, etc., via transmission electron microscopy, the state must be "frozen" (vitrification). Vitrification (picture & link), which developed by Nobel Prize winner Jacques Dubochet, allows the amorphous freezing of the liquid phase by extremely rapid cooling in liquid ethene. This suppresses the crystallization of the liquid. The finished TEM sample is therefore amorphous and allows the materials to be examined without itself making a significant contribution to the contrast of the image data.



NobelPrize.org, Schema von J. Jarnestad/
The Royal Swedish Academy of Sciences

Motivation

1. Aim of the preparation →

Conservation of the structure

2. Challenges

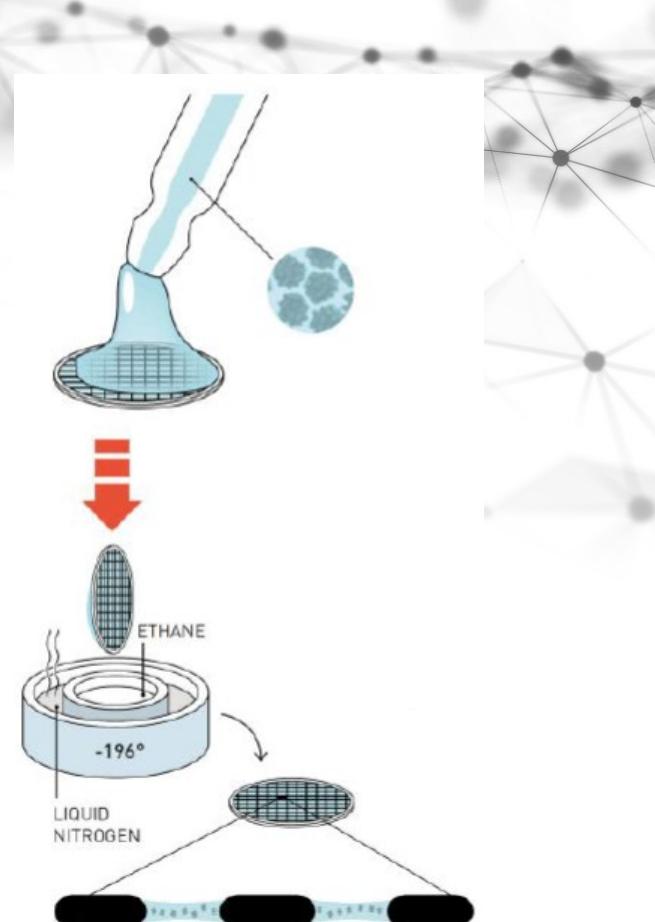
→ Dehydration (drying) or exchange of the liquid changes the structure

3. Advantage of vitrification

→ fast freezing of the sample
→ Structure remains intact

4. Factors influencing quality

→ Temperature of liquids
→ the Plunging speed
→ thickness of the sample



NobelPrize.org, Schema von J. Jarnestad/
The Royal Swedish Academy of Sciences

Task

Development and construction of a Plunge Freezer

A device for freezing suspension and biological samples into an amorphous state for TEM analysis

Aims

- Low cost and semi-automatic version
- Design, production, assembly, test



Transmissionselektronenmikroskop
(TEM)

Requirements

- Plunging speed
- Dabbing the sample
- Temperature resistance
- Temperature monitoring

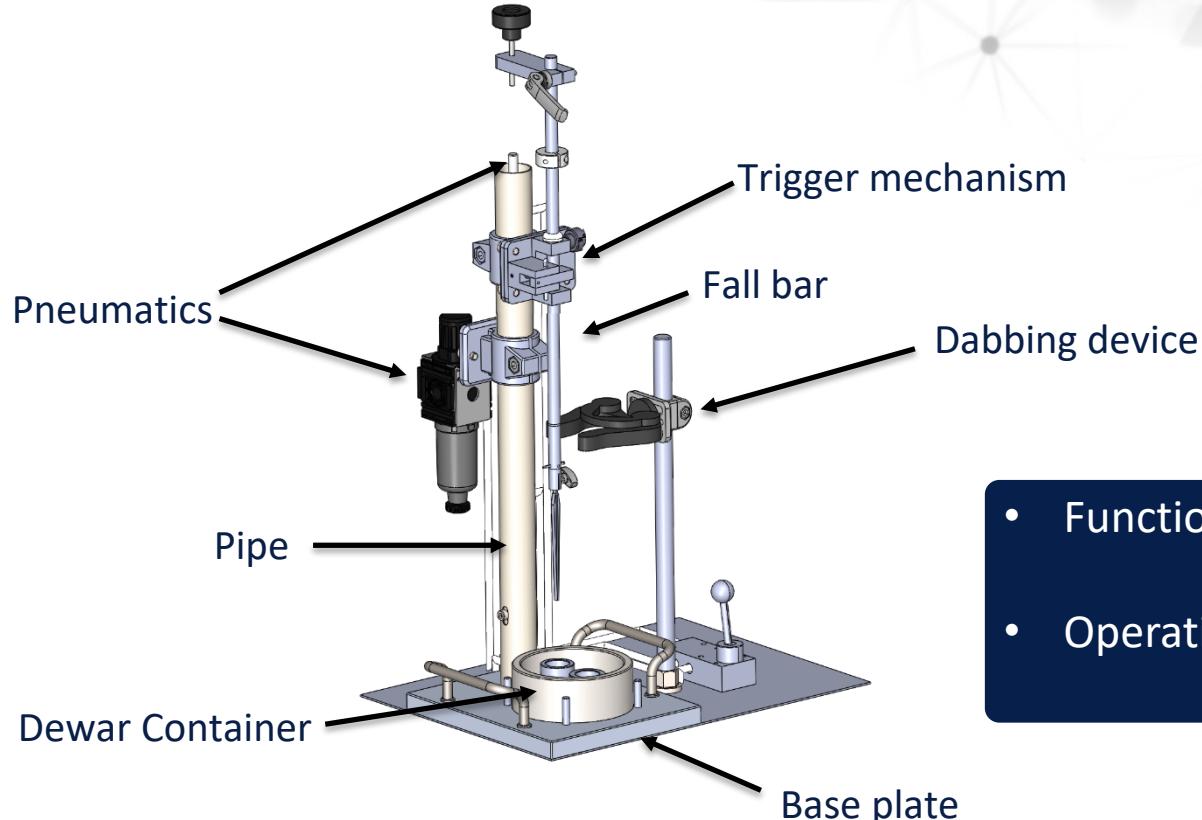
- Transport container
- Holder for the Dewar
- Removable forceps
- geometric size

- Transportable
- Cost-effective
- Reproducibility



Implementation and function of the components

Basic framework



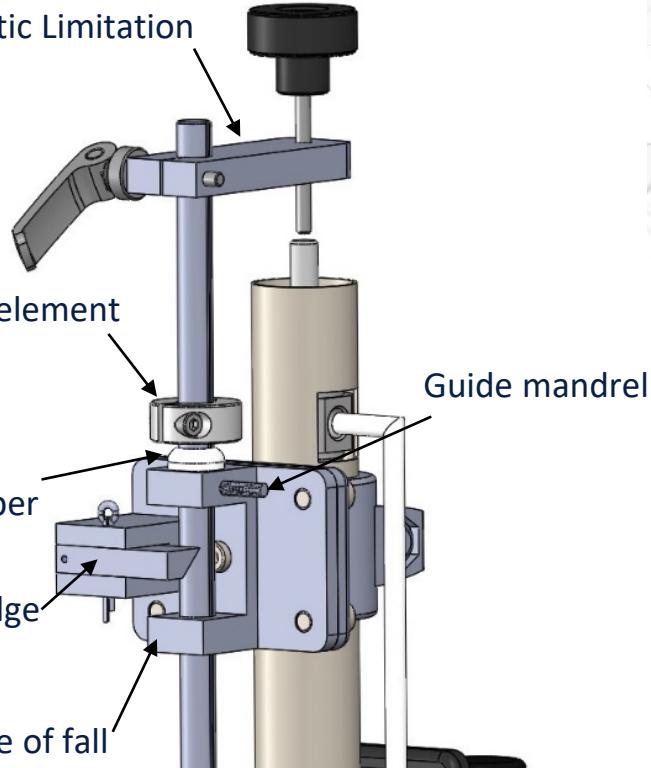
- Functional parts developed
- Operating elements purchased

Fall mechanism

Fall bar

- goes through a duct
- secured against twisting

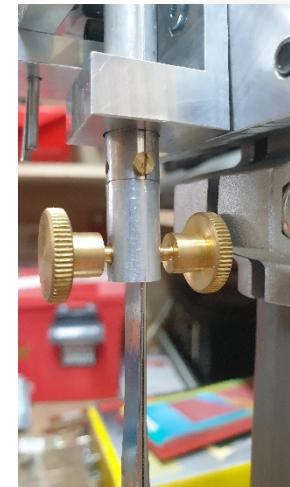
Pneumatic Limitation



Forceps

- connected via connector
- removable
- 90° rotatable

Forceps



Trigger mechanism

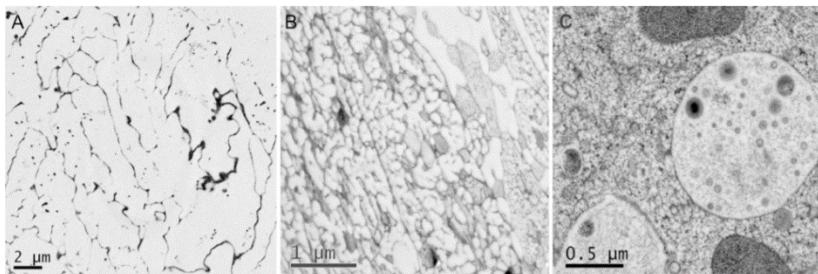
- Release by Bowden cable
- secured by wedge in notch

Swab / gripper

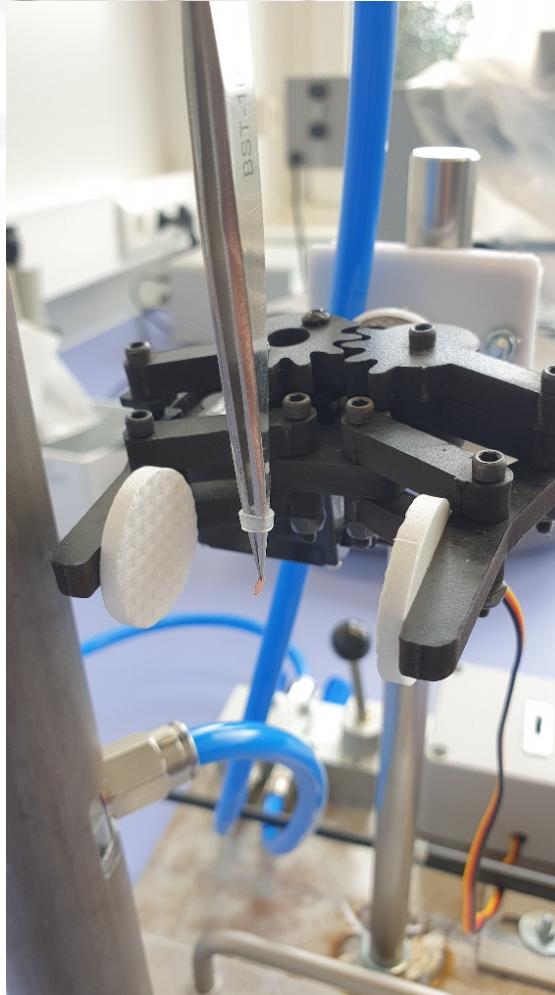
intended to remove the remaining liquid

- lower price segment
- simple and compact control
- sufficient strength
- limited operating times
- free and simple software

Because of the long development and construction time required, it was bought

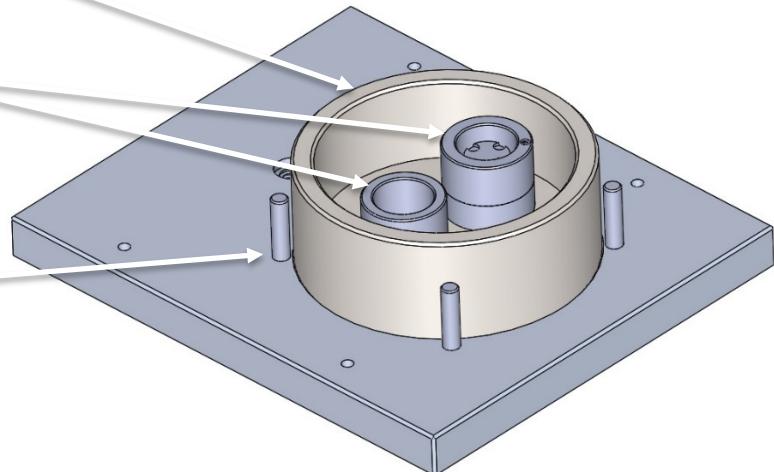


Quelle: Rudolph Reimer: Kryotechniken. Springer Verlag, 2015, S.160



Dewar vessel

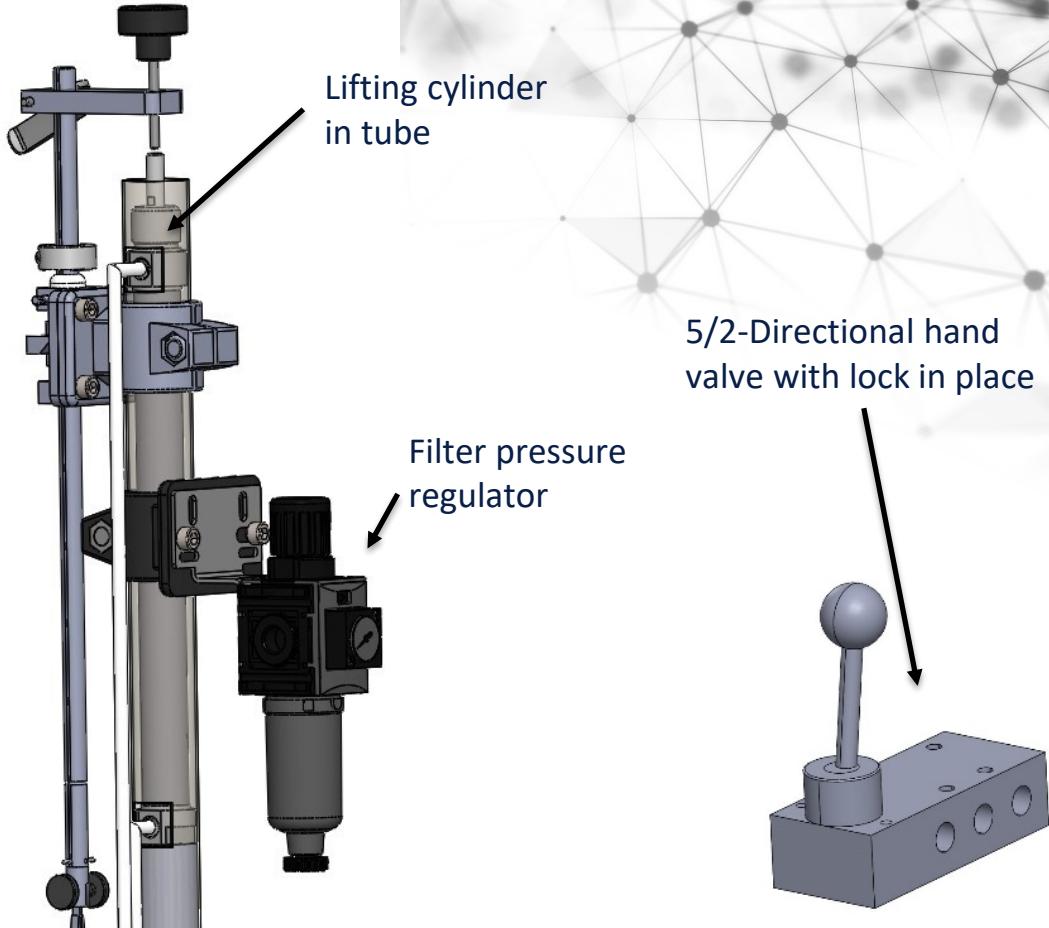
- Contains liquid nitrogen
- Two small vessels with liquid ethene
 - one for Plunging
 - a transport container
- no matching commercial parts
- Vessels made of steel and aluminium
- stands in notch and secured with grub screws
- not double-walled; it is self-produced



Pneumatics

Function:

- Drive the fall bar down and lift it into the basic position
- Costs were saved by rudimentary construction



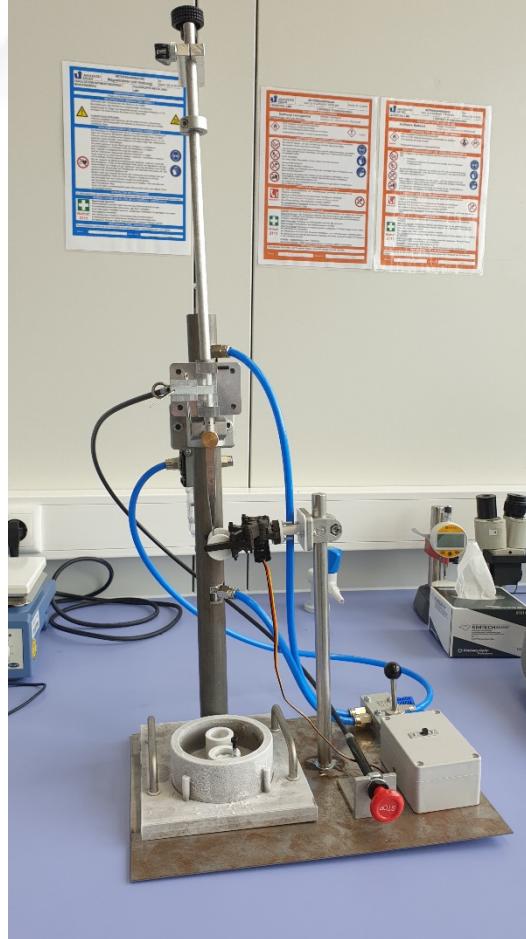
Summary

Challenges

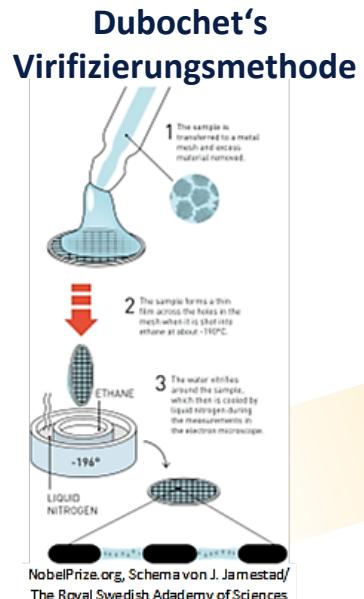
- Corona induced delays
- Degree of automation
- Budget
- Speed of fall

Evaluation

- + ready to use device
- + Cost-effective
- only limited Automation
- missing temperature recoding



Thanks for your Attention



Plunge Freezer

**Transfer-Halter
(oder Cryo-Halter)**



Plunge Freezer



Transmissionselektronenmikroskop (TEM)