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University of Basel
Swiss Nanoscience Institute

SNI SWISS NANOSCIENCE INSTITUTE
EINE INITIATIVE DER UNIVERSITÄT BASEL UND DES KANTONS AARGAU

NANO IMAGING LAB

Newsletter

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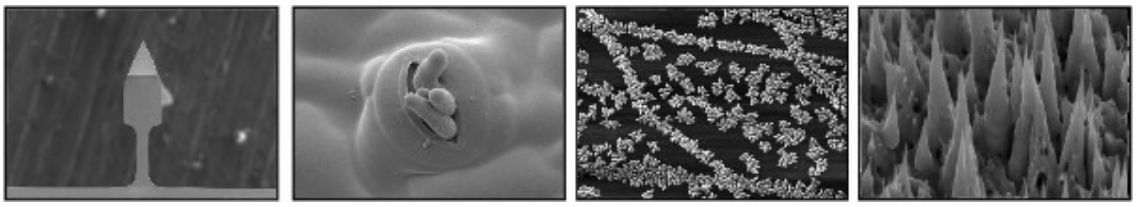


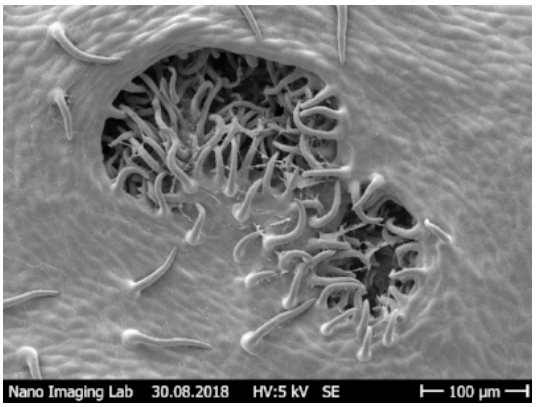
Freeze drying is in use at the Nano Imaging Lab

Freeze drying as a method to prepare samples is well known since the 1970's. There are some advantages in contrast generation in an electron microscope compared to the inspection of fully hydrated samples embedded in vitreous ice. Freeze drying was never considered to preserve ultrastructure well, because at that time ultra-rapid freezing was not applied before freeze drying. Freeze drying was given up, because researchers did not see any advantage in adding a drying step with the risk of introducing artefacts, if samples could be observed fully hydrated.

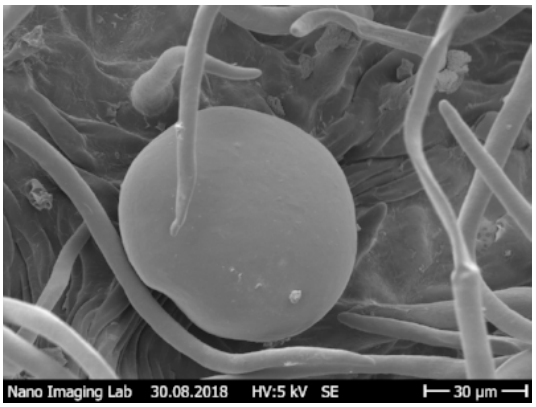
Water has the density of 997 kg/m³. This does not differ much from the density of living cells with 1050 kg/m³. Therefor cells embedded in vitreous ice do not show much contrast based on density. Freeze dried cells in vacuum (density of air: 1.2041 kg/m³, reduced by a factor 10^{-6}) show a tremendous density difference. Therefor the contrast of a sample in TEM is expected to be considerably better after freeze drying compared with fully hydrated sample in amorphous ice.

Cells ultra-rapidly frozen in PBS buffer show salt crystals after freeze drying. To avoid this, buffers with vacuum-volatile components have to be used.

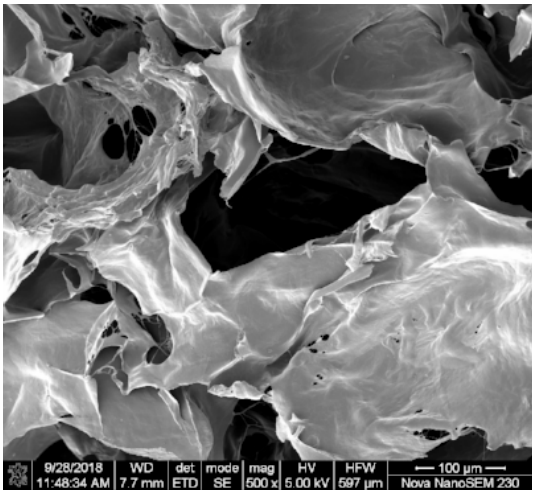
First pictures of freeze dried material are depicted below:



Oleander leave (photo: Evi Bieler)



Sage leave (photo: Evi Bieler)

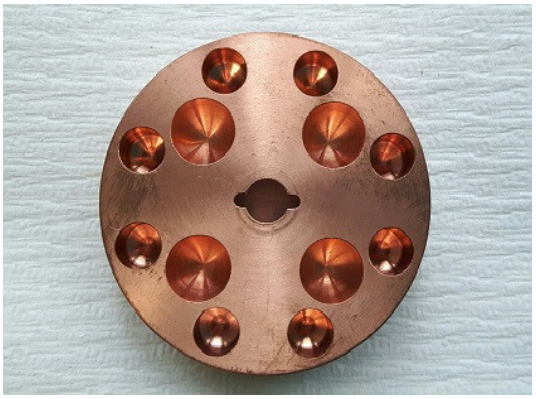


Collagen scaffold (photo: Daniel Mathys)

The NI-Lab is establishing the freeze drying method again. We combine ultra-rapid freezing followed by freeze drying. The treatment of the samples with a volatile buffer before freezing seems to be a breakthrough. We developed the preparation chain for TEM-grids, SEM supports and cover glasses for AFM use.

These three different specimen holders are available :

1. regular holder
2. holder for TEM-grids
3. holder for round 12mm-coverslips



Leica Tic3X workshop

On 6th of September 2018 the NI-Lab organized a workshop in collaboration with Leica Switzerland to demonstrate the Argon-plasma cutter and polisher type Leica TIC 3X and the shaping machine EM TXP.

This is a perfect preparation line to first mechanically shape samples (EM TXP) and then polish the surfaces of interest with Argon-plasma before imaging in a SEM. The aim is to see cross sections of all kinds of material and to identify surface elements. Even grain distribution in alloys and pure solids can be imaged after that manner of preparation.

Twelve participants, two Leica representatives and the NI-lab team met at 8:30 for coffee and croissants. Then there was a theoretical introduction to ion etching by Dr. Wolfgang Grünewald followed by challenges and characteristics of specimen preparation. During the afternoon the two machines could be tested intensely by the participants. A round table discussion and closing remarks brought the workshop to an successful end.
It was a fruitful day with a lot of opportunity to exchange experiences and learn new strategies from the expert.

BoVitis

BoVitis will be the name of the follow up project of Vitifutur, which will end in 2020. For three further years (2020-2023) leading research institutions of the Oberrhein-Region will work together within the frame of InterregV to fight vine diseases.The Nano Imaging Lab will also continue to be a part of this trinational project between Alsace, Baden-Württemberg and Northwestern Switzerland.

The wine industry is of great economic importance for the transnational region of the Upper Rhine. However, in many years the economic efficiency of farms on both sides of the Rhine has been considerably been impaired by sour rot in the berries.

The sour rot is caused by the fungus *Botrytis cinerea*, which colonizes ripening berries and decomposes the pulp with the help of enzymes.
Botrytis infects the berries via microcracks on the berry surface using a germination tube to intrude into the fruit. It rapidly forms masses of fungal spores, that detach and are transported to other berries by the wind.The fungus develops rapidly in warm and humid conditions: at temperatures above 20°C it germinates after a few hours and begins to colonize the berry.
An innovative approach to avoid quality loss due to Botrytis, is the use of resistant grape varieties. Observations on various kinds and wild species show that some are less susceptible or even immune to Botrytis. This reduced susceptibility may be due to the properties of the berry surface. The structure of the wax layer and the physical-mechanical properties of the berry skin play a major role here. According to preliminary studies, the nanostructure of the wax crystals on the surface differs between grape kinds.
The wax surface has a great influence on the moisture conditions on the berries and thus influences the germination readiness of Botrytis spores and, depending on their structure and thickness, hinders the colonization of the berry.
Another barrier is the berry skin, which, depending on its physical-mechanical properties, prevents the fungus from penetrating. In addition to these structural barriers, defense mechanisms are activated by the plant as soon as it detects a infection.
The complex response of the cells in the outer berry tissue is known but not yet thoroughly investigated.
The developed barrier of wax crystals, robust berry skin and the activated defense response offer the potential for breeding new varieties with resistance to Botrytis. So far these mechanisms in berries have not been studied in detail.
There is an extensive collections of varieties and wild varieties of vines of great international reputation, that could be used as a resource for Botrytis resistance, but this

potential is still insufficiently exploited.
The project BoVitis will investigate the genetic basis of resistance to Botrytis and the stability of the berry surface, which is specific for each species, with the aim to be able to cultivate resistant grapes.
The development of scientifically based methods for the sustainable management of Botrytis is an extraordinary challenge, that requires joined research activities on all sides of the Upper Rhine.

The Nano Imaging Lab will be involved in revealing the bionics of the berry surface.
Since Cryo SEM is very well established in our lab, we will use this technique to investigate and image the ultrastructure of berry surfaces. Cryo SEM is the only way to depict the solvent sensitive wax structure.

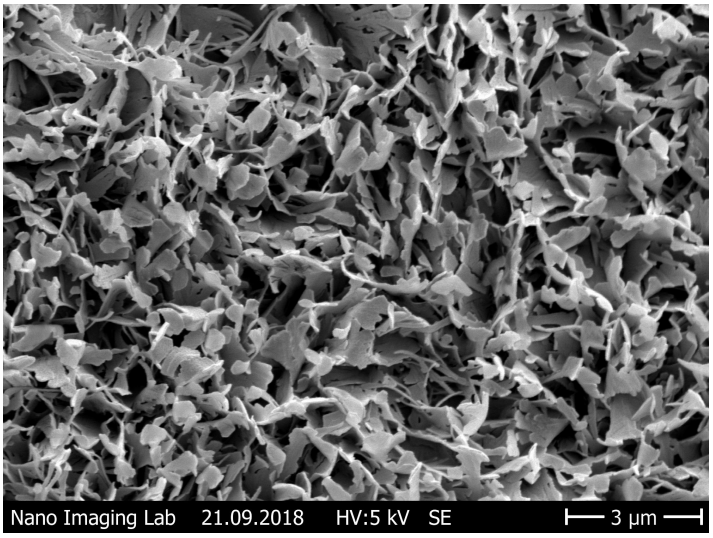


Image of an intact wax layer of a Cabernet Sauvignon berry
(photo: Evi Bieler)

http://nanoimaging.unibas.ch