TECHNICKÁ UNIVERZITA V LIBERCI

Nové možnosti rozvoje vzdělávání na Technické univerzitě v Liberci

Specifický cíl A2: Rozvoj v oblasti distanční výuky, online výuky a blended learning

NPO_TUL_MSMT-16598/2022

Microscope, microscope slides

Ing. Bc Monika Vyšanská, PhD.

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Národní plán obnovy

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

Development of light microscopes [1]

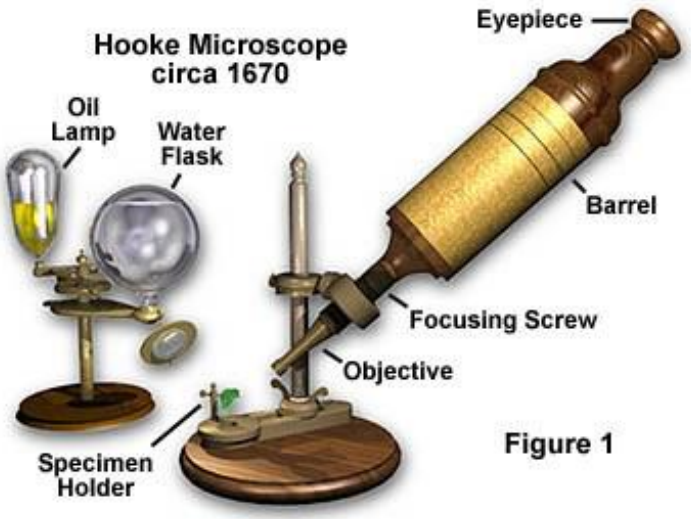


Figure 1

1670

1850

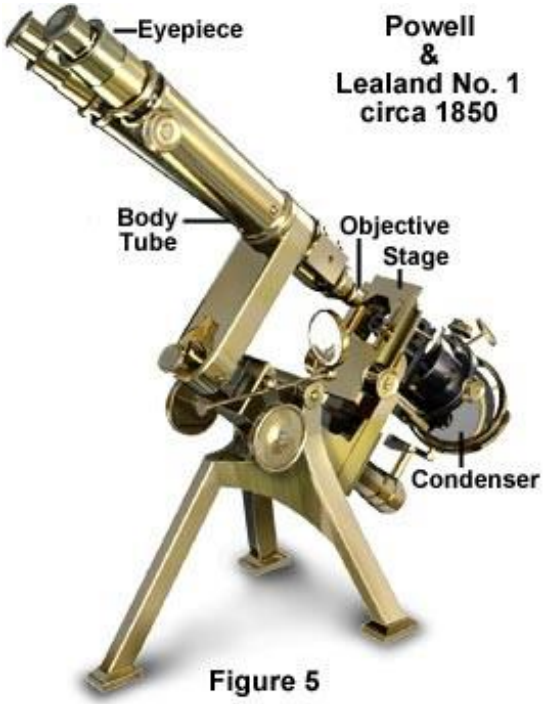


Figure 5

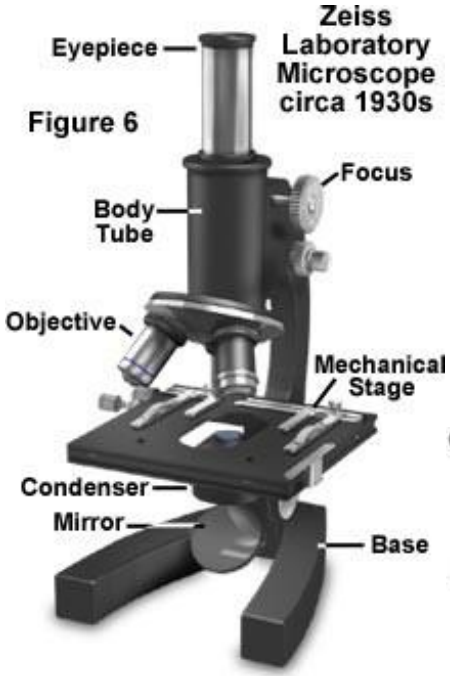


Figure 6

1930

1998

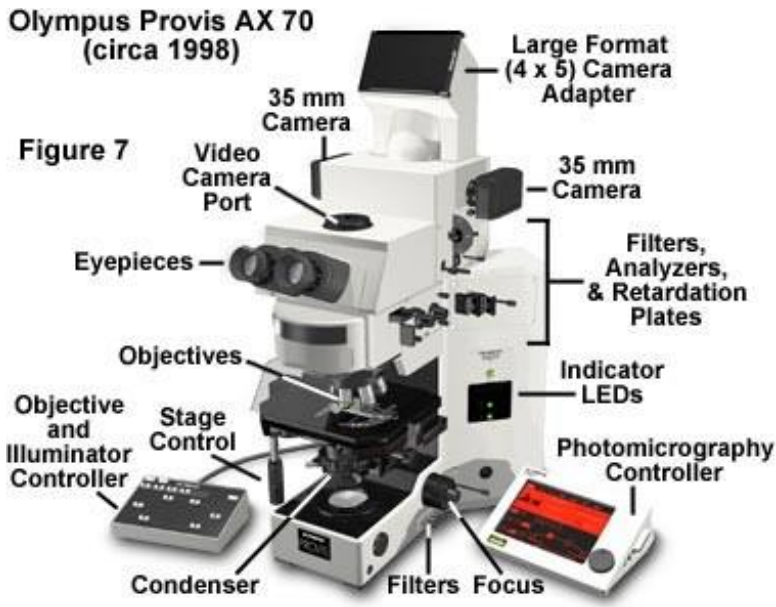


Figure 7

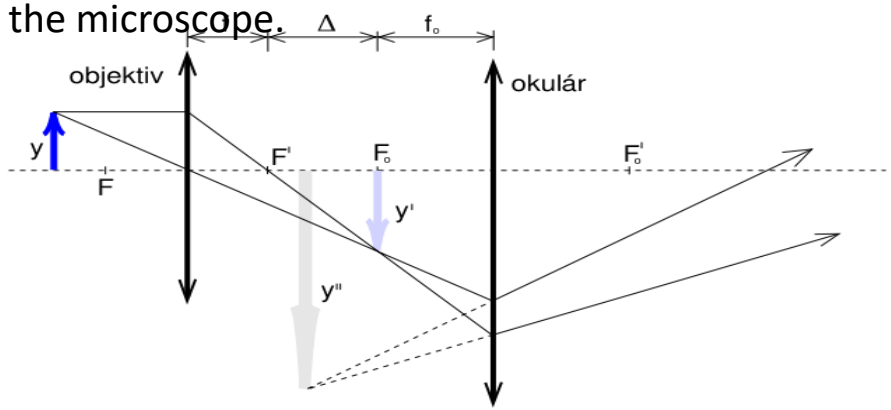
Optical microscopy [2]

- allows us to observe things that our eye can no longer distinguish (the resolving power, i.e. the ability to distinguish 2 points next to each other, is 0.25mm in humans).
- the resolution of light microscopy is approximately **0.25 µm**, which is due to the wavelength of the radiation that passes through the microscope (in this case **light**, a stream of photons), but also to the properties of the objective (see below).
- the resolving power of light microscopy is therefore **1000 times greater** than that of the human eye.
- the maximum useful magnification that can be achieved in light microscopy is up to **2000** for special microscopes. For a higher level of detail, electron or atomic force microscopy is used.

Light microscope [2]

A light microscope consists of **three connected optical systems**: the illumination system (condenser), the objective and the eyepiece.
The illumination system (in the picture under number 7) is used to illuminate the slide, most often against the direction of observation. The preparation illuminated in this way must be partially transparent.
The lens (pictured under number 3) is a set of lenses with a very short focal length, which work together as a continuous lens to show the object (slide) inverted, real and magnified. The resulting image is projected between the focus of the eyepiece and the lens.
The eyepieces (pictured under number 1) are also made up of a set of lenses that function as a continuous lens. In this case, they act as a magnifying lens that produces an apparent, straight and magnified image.

It also has a **mechanical part** that completes the microscope.



1) eyepieces, 2) revolver, 3) lenses, 4) macro screw, 5) microscrew, 6) table, 7) light source, 8) condenser and aperture, 9) cross shift

Light microscope [2]

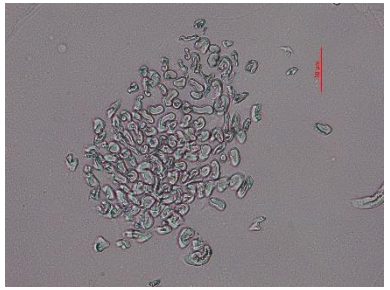
The components of the mechanical system are:

- **a table with a slide** through which the object plane passes,
- **cross shift**, which allows the slide to be shifted in the subject plane (in practice, it allows the entire slide to be overlooked),
- **macroscrew and microscrew**, which allow coarser and finer scrolling of the stage up and down - changing the distance between the object plane and the lens allows focusing on a given structure,
- **a revolver** that allows the lens to be changed (usually to a different magnification lens) so that the distance between the subject plane and the lens is maintained,
- **a tube**, separating the lens from the eyepiece, which prevents the entry of unwanted light rays from the external environment,
- **eyepiece focusing ring**, which occurs only if the microscope has two eyepieces, one for each eye: in this case, the focusing ring is located on only one eyepiece and allows correction of the difference between the user's eye defect by moving the optical system of one eyepiece relative to the optical system of the objective,
- **rubber eyepiece of eyepiece holder**, which serves to fix the eyes at the correct distance from the eyepiece lenses.

1) eyepieces, 2) revolver, 3) lenses, 4) macro screw, 5) microscrew, 6) table, 7) light source, 8) condenser and aperture, 9) cross shift

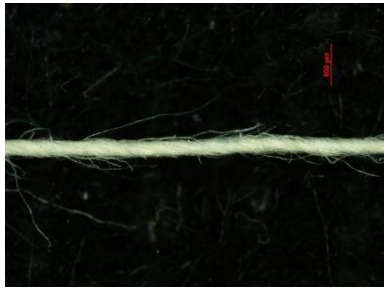
Imaging methods in light microscopy [2]

The transmitted light method



- Light **passes through** the object being observed (in transmitted light) and a set of two connected lenses produces the actual, magnified and inverted image that we observe through the eyepiece.
- The object has dark outlines and is located in the **light field** as opposed to the dark field where the object is light and is located in the dark field.

Dark field method



- The object is illuminated by a condenser so that only the **peripheral**, very oblique light rays enter the plane of the object, while the central ones are not absorbed and therefore do not apply at all in imaging.
- The subject is therefore illuminated from the sides and the rays are reflected and refracted from it, so light that is scattered by the subject enters the lens. The object appears to glow against a dark background and is therefore very visible.
- When observing in a dark field, those parts of the object shine on a dark background where there is a sufficient difference in the passage of light through the observed object, such as its edges.
- The method is used to **observe small objects and their surface structures**, e.g. protozoa, bacteria, plant tissues, pollen grains, etc.

Imaging methods in light microscopy [2]

Phase contrast method

- The method is used to **highlight the contrast** of small phase objects where the details do not differ from the surroundings by absorption, but cause a phase change.
- **It converts differences in the phase shift** of light passing through different parts of an object that we cannot see into differences **in intensity** that we can observe.

Ultraviolet microscopy

- It uses UV radiation as a light source, which is characterized by a shorter wavelength than visible light in the range of approximately 400 - 10 nm, which increases the resolving power of the microscope.

Infrared microscopy

- Infrared radiation, whose wavelength ranges from 760 nm to 1 mm (in IRM it is mainly used in the wavelength range 760-1100 nm, the so-called near field), is invisible and has significant thermal effects (the human body perceives it as radiant heat). It penetrates some objects more easily than visible light and can therefore be used to study more powerful preparations.

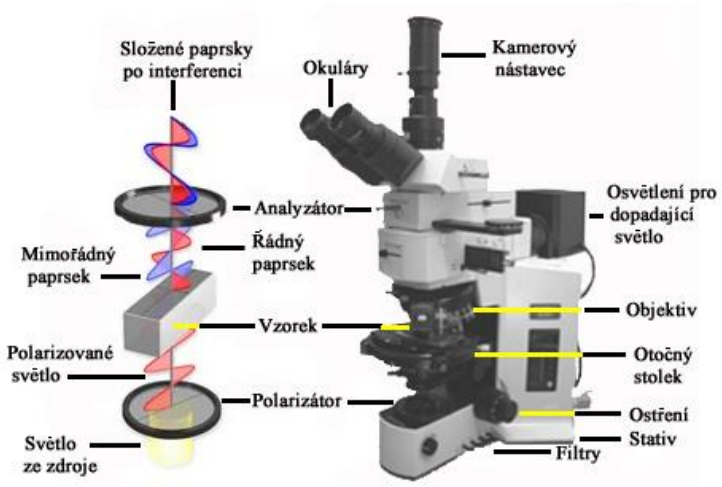
Imaging methods in light microscopy [2]

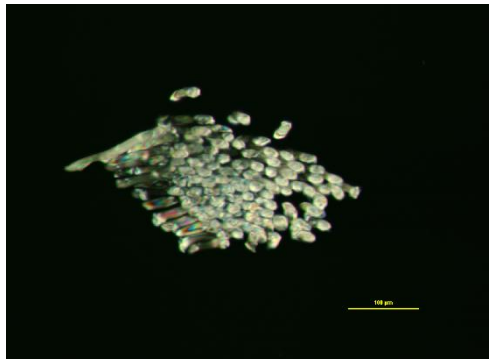
Inverse microscopy

- Inversion microscopy usually uses visible light.
- **Tracking objects not sealed in the confined space between the slide and cover slip.** It allows observation of objects in thick-walled containers (e.g. petri dishes), or objects floating freely in liquids in different dishes.
- **The light source is located above the microscope stage**, the object is placed in a dish on the stage, the objective is placed under the stage and the eyepiece is usually placed behind a prismatic or planar reflector (mirror) in a direction suitable for comfortable observation.

Polarization microscopy

Uses the optical activity of the slide under examination (i.e. the ability of the slide to bend the plane of polarized light).





The optical path of light includes **two polarizing filters** that have crossed polarization planes. The rays of the light source first pass through the so-called **polarizer** (formerly a crystal of a double-breaking substance, now a plastic polarizing filter) - here the polarized light is formed, then they pass through the observed object, the normal lens, then through the second polarizing filter called the **analyzer** and then through the eyepiece.

Imaging methods in light microscopy [2]

Interference microscopy

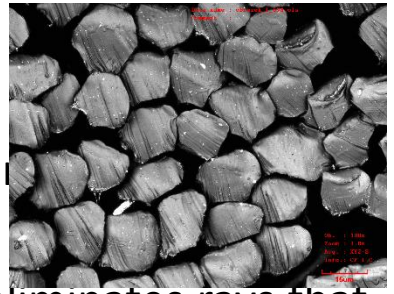
- It works on the principle of comparing one beam after passing through the air and another after passing through the sample.
- The two-beam interference microscope consists of an optical microscope and an interferometer. Using a set of interferometer, condenser and Wollaston prism, the beam coming to the slide is split into two. One passes through the specimen, which changes its phase. The other passes outside and therefore serves as a reference.
- The method works on the principle of the dark field - with a suitable interferometer setting, the incoming beam is composed of **two beams** that are shifted by half a wavelength relative to each other.
- **If the sample is not placed**, the rays are "cancelled out" and **nothing can be seen** in the field of view.
- If one beam passes through the slide and its wavelength changes, the total wavelength of the resulting beam will also change and the image of the sample will no longer be "dark" in the interferometer or eyepiece.

Imaging methods in light microscopy [2]

Fluorescence microscopy

Fluorescence - the process whereby a substance, **after absorbing a quantum of radiation, emits radiation of a longer wavelength.**

- Primary fluorescence - the substance in the preparation itself acts as a fluorochrome (e.g. chlorophyll)
- Secondary fluorescence - a fluorochrome is added to the sample to label initially non-fluorescent structures (e.g. immunoglobulins with a fluorescent marker in immunohistochemistry).
- Fluorescence microscope - an optical microscope with a strong radiation source (usually mercury or xenon lamps), a monochromator (transmits only one wavelength - colour of light) and a condenser.
- This microscope is used, for example, to detect individual proteins or molecules in a cell.



Laser scanning confocal microscopy

- The light emitted by the laser towards the slide first passes through a narrow slit - poi concentrates the beam into a single point in the sample.
- From there, the beams pass into the lens, where a second aperture is placed, which eliminates rays that do not come from that point in the sample.
- The rays that do pass through are detected on the photomultiplier tube and the position and shape information is sent to the computer.
- In this way, enough points are analyzed to cover the entire sample so that the computer can produce the resulting image.

Microscopy workflow (for refresher ☺) [3]

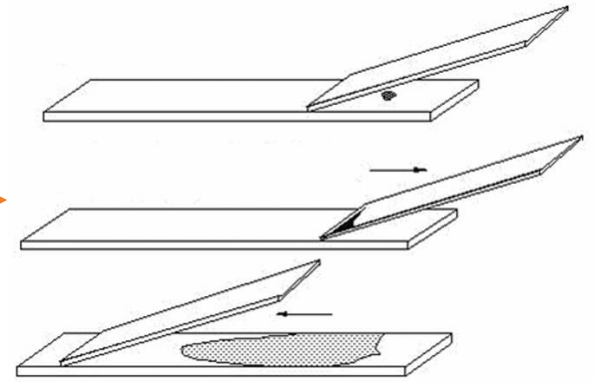
1. Place the microscope on a flat surface (Caution: when carrying the microscope, grasp the tube holder with one hand and support the tripod with the other hand)
2. Connect the microscope to the mains and adjust the illumination, or adjust the position of the mirror to make the field of view as bright as possible.
3. Mount the slide on the microscope stage (the slide must be at the bottom).
4. Adjust the smallest magnification of the objective.
5. Using the micrometer screw, reduce the slide-to-object distance to the minimum (check from the side!).
6. Using the eyepiece, observe the field of view and at the same time focus with the micrometer screw by slowly increasing the lens-preparation distance to the most suitable position (rotate in the direction of the eyepiece). Using the cross-shift screw, move the slide along the stage to the centre of the field of view.
7. Sharpen the search image with the micrometer screw only.
8. If a higher magnification is needed, change the lens using the turret changer and sharpen only with the micrometer screw.
9. When observing, remember to sharpen the objects with the micrometer screw to observe the different planes of the object under study.
10. The image quality can be influenced by adjusting the position of the condenser and closing the aperture.
11. Plot the observed object, describe it and record the magnification at which the observation was made.
12. When the observation is finished, do not forget to remove the slide from the microscope.
13. When the work is finished, turn off the microscope, cover it with a cover and store it carefully. If dirty, wipe gently with a soft cloth, never touch the optics with your fingers.
14. Clean up the work area after yourself.

Microscopic slides [4]

- **Temporary (native**) - we usually make, explore, describe and then cancel, the closing environment is most often water
- **Permanent** - can last for decades, collections of permanent slides of objects from

the site in permanent media (e.g. Canadian balsam)

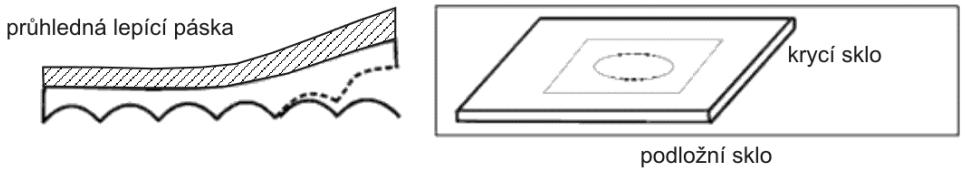
Division of preparations according to the method of preparation:



Coating and spreading - the sample (e.g. soft tissue or suspension of particles) is

smeared or spread onto a glass substrate.

- Microrelief and adhesive slides - used to study the surface or surface layers, they allow to observe considerable details of the object surface. The principle is the application of a thin layer of a rapidly solidifying transparent substance (e.g. colourless nail polish, glue, ..) on the surface of the slide. After drying, the impression is removed with transparent adhesive tape and transferred (glued) to the slide. The relief type of slide is used, for example, to study the skin of a leaf.



- Cross-sections - can be native or permanent
- Permanent cuts - the cut must be fixed immediately

(by heat, drying, deep freezing, freeze drying, freeze substitution or chemical fixatives) and embedded in a block (embedding compound - gelatine or celloidin, most often paraffin). Finally, the blocks are cut into ultra-thin sections (thickness of a few µm) using a special device - a microtome (see figure below).

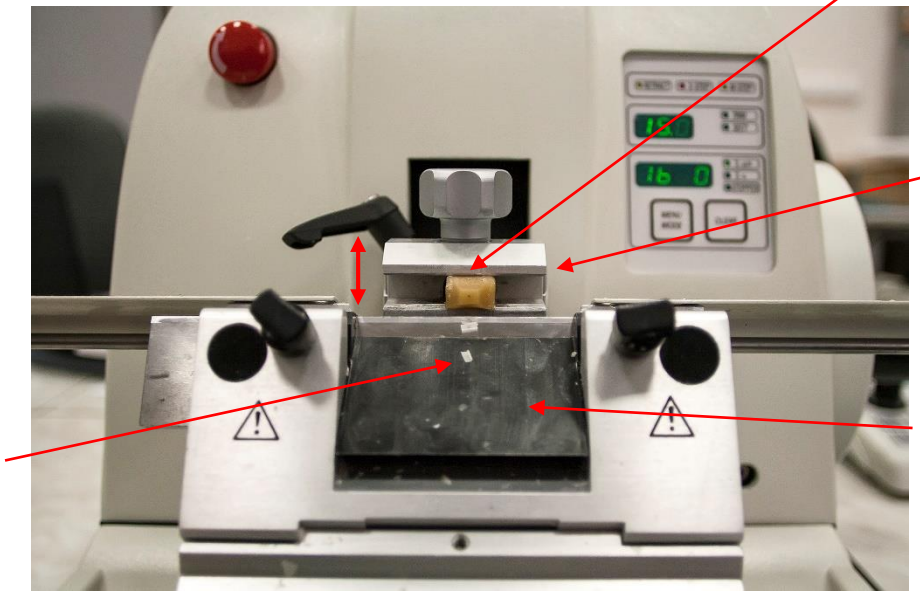
IN 46-108-01/01 Recommended procedure for creating cross sections. Soft and hard cuts [5]

Description:

Creating cross-sections of length textiles (fibre, fibre bundle, silk, yarn, etc.) and flat textiles (fabrics, knits, non-wovens, etc.). The textile is cast into a medium which, when solidified, forms a block from which micrometric cuts are separated using a special technique.

Microtome

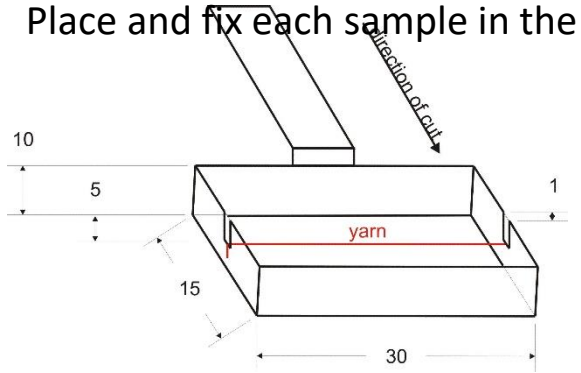
(e.g. sliding, rotary, manual or automatic); and knives (depending on the type of microtome e.g. steel knife 16cm/c-profile for soft cuts, glass knives for hard cuts, diamond knives, etc.).



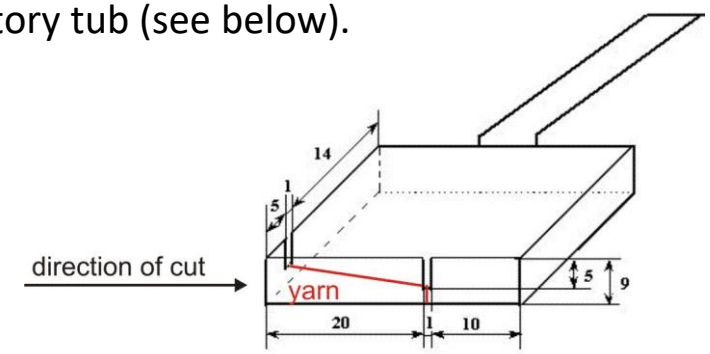
IN 46-108-01/01 Recommended procedure for creating cross sections. Soft and hard cuts [5]

Principle:

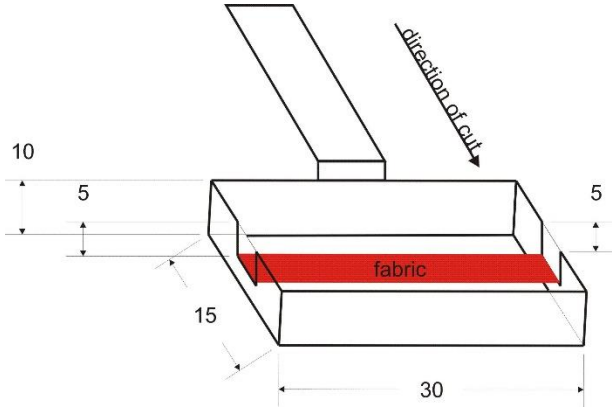
- A minimum of 30 samples shall be taken from the skein of yarn whose fineness is closest to the average of ten measurements.
- Fixation of the 30 samples in three steps on three consecutive days: with very dilute glue (Spolion 8), with slightly dilute glue (Spolion 8), with undiluted glue (Spolion 8).
- Place and fix each sample in the laboratory tub (see below).



Bathtub: perpendicular cuts through the length formations



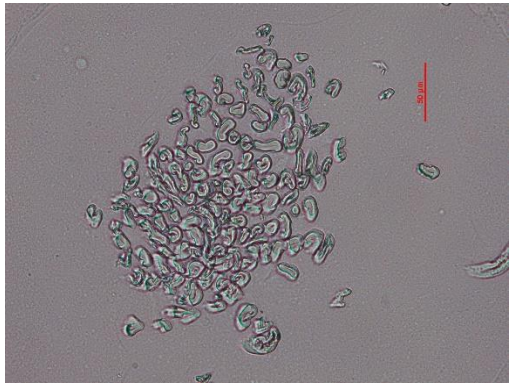
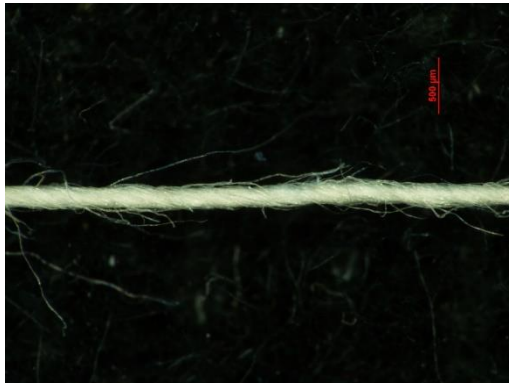
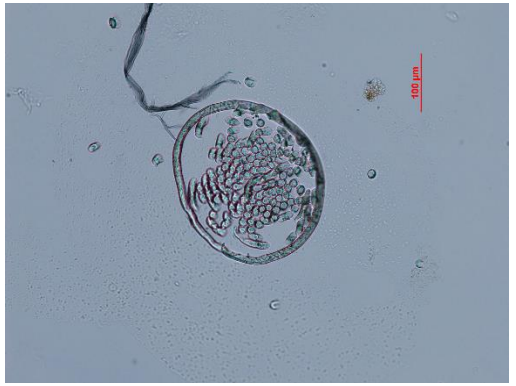
Bathtub: oblique cuts with length formations

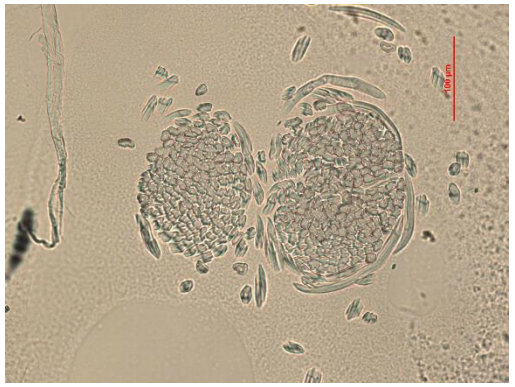
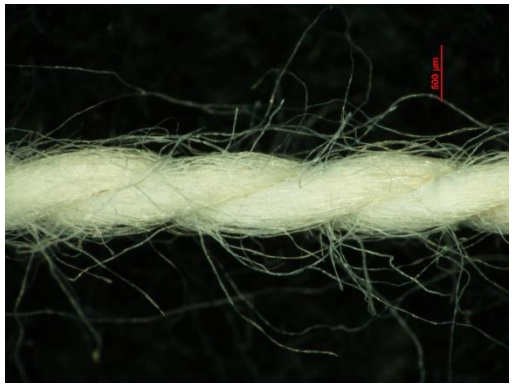
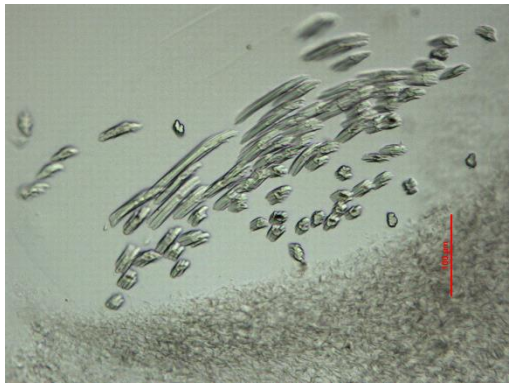


Bathtub: oblique cuts with length formations

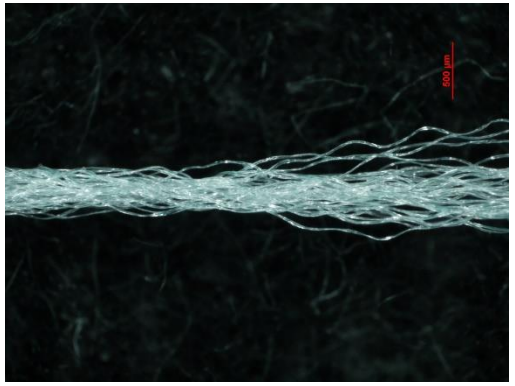
- Pouring the sample with a 1:1 mixture of paraffin and wax.
- Cooling of samples in baths (refrigerator).
- Forming a pyramid around the sample (removing excess wax and paraffin).
- Cutting with a microtome knife using a microtome (see previous slide).
- Removing individual sections with tweezers on a xylene slide (dissolves wax and paraffin).
- Microscopy without coverslip using a light microscope - transmitted light.

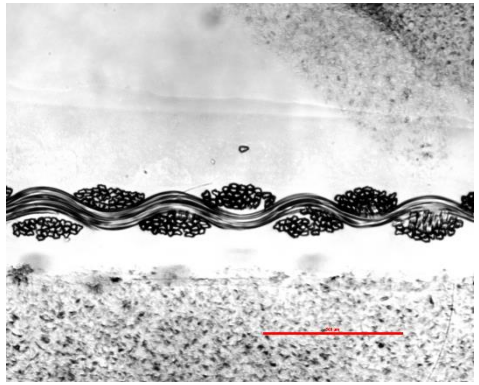
Sample images of basic fabrics (sections, longitudinal views)

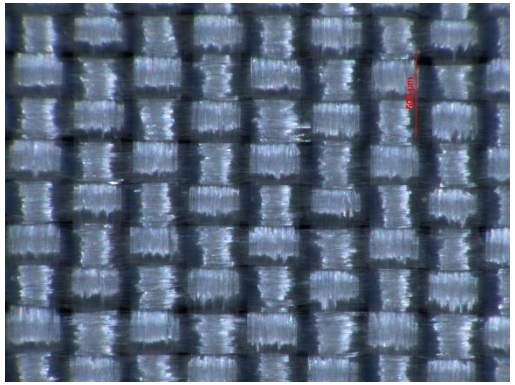




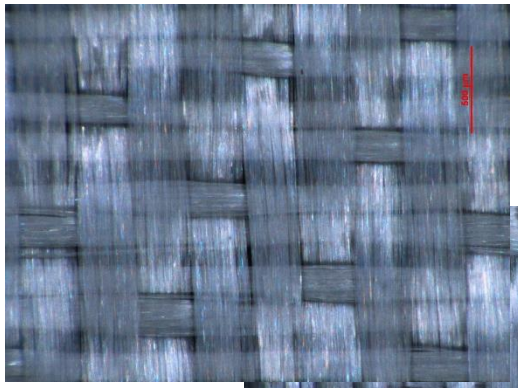




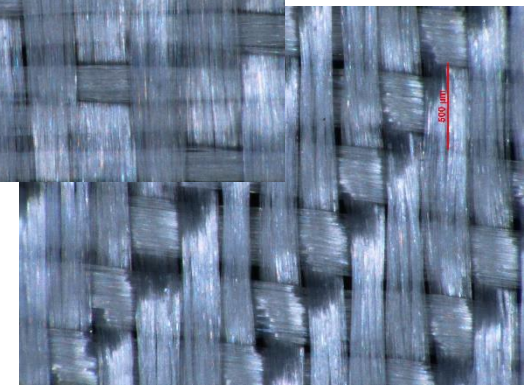
Sample pictures of basic fabrics (cuts, longitudinal views)

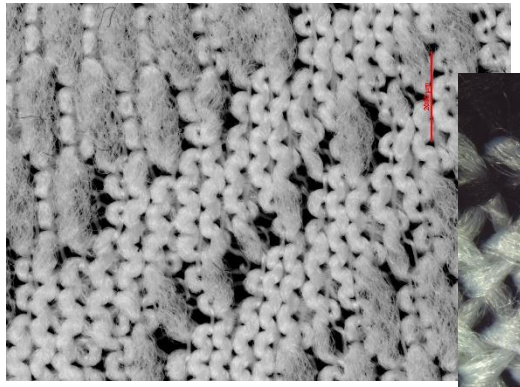


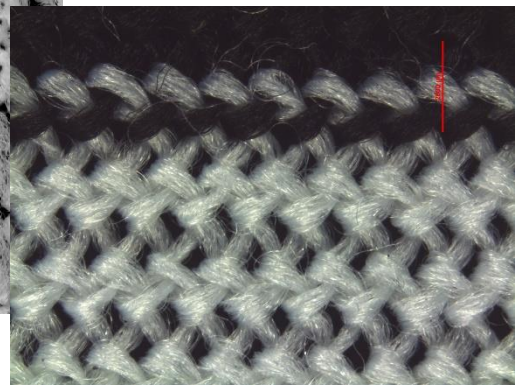






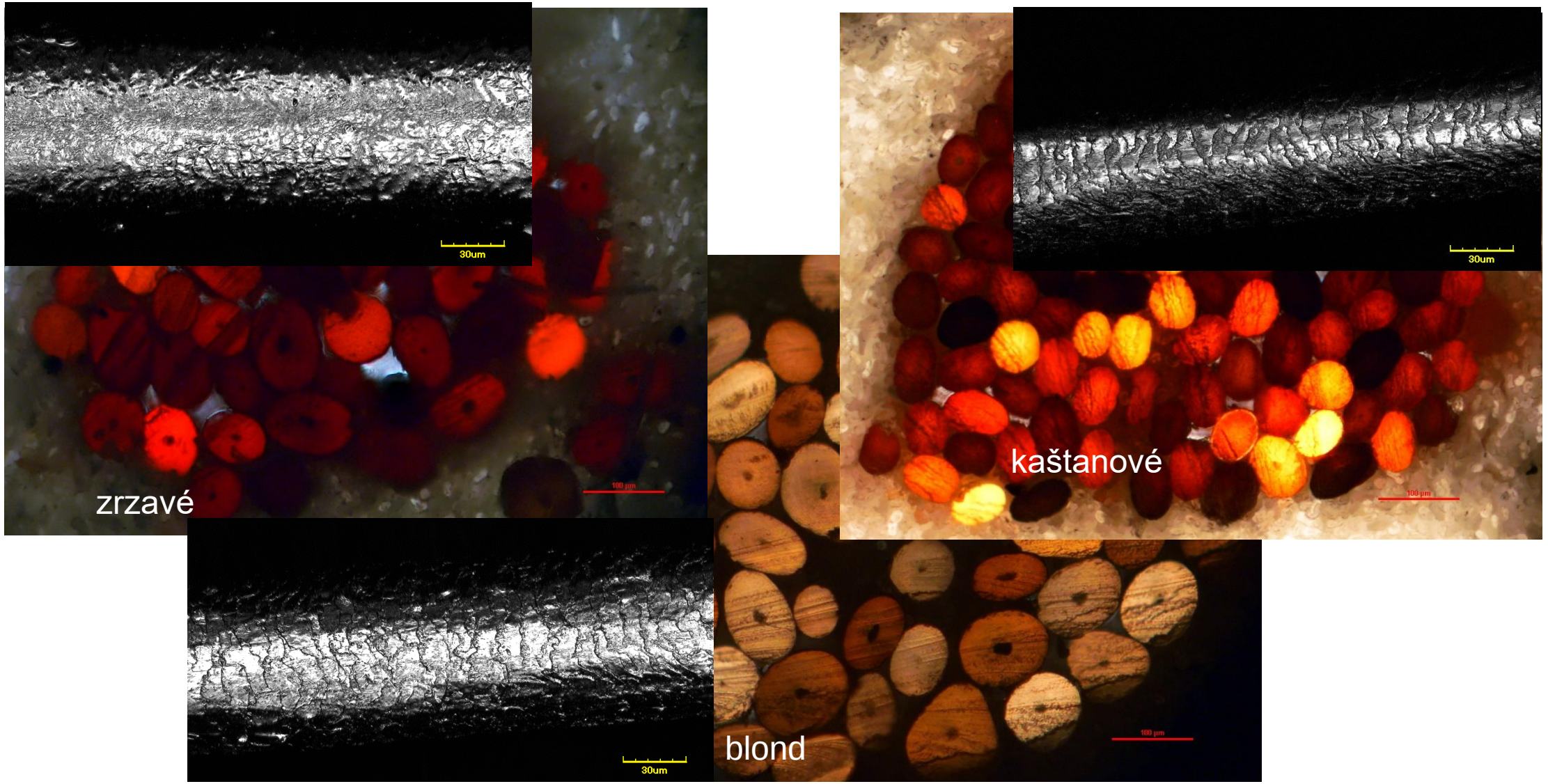




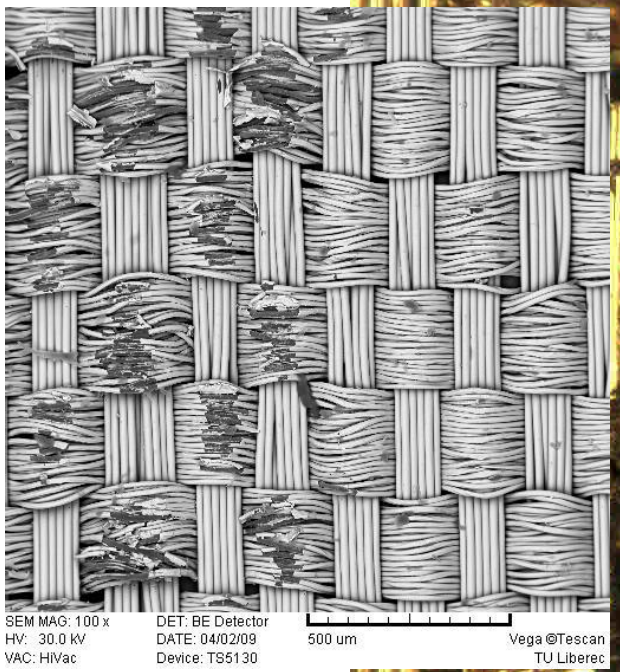


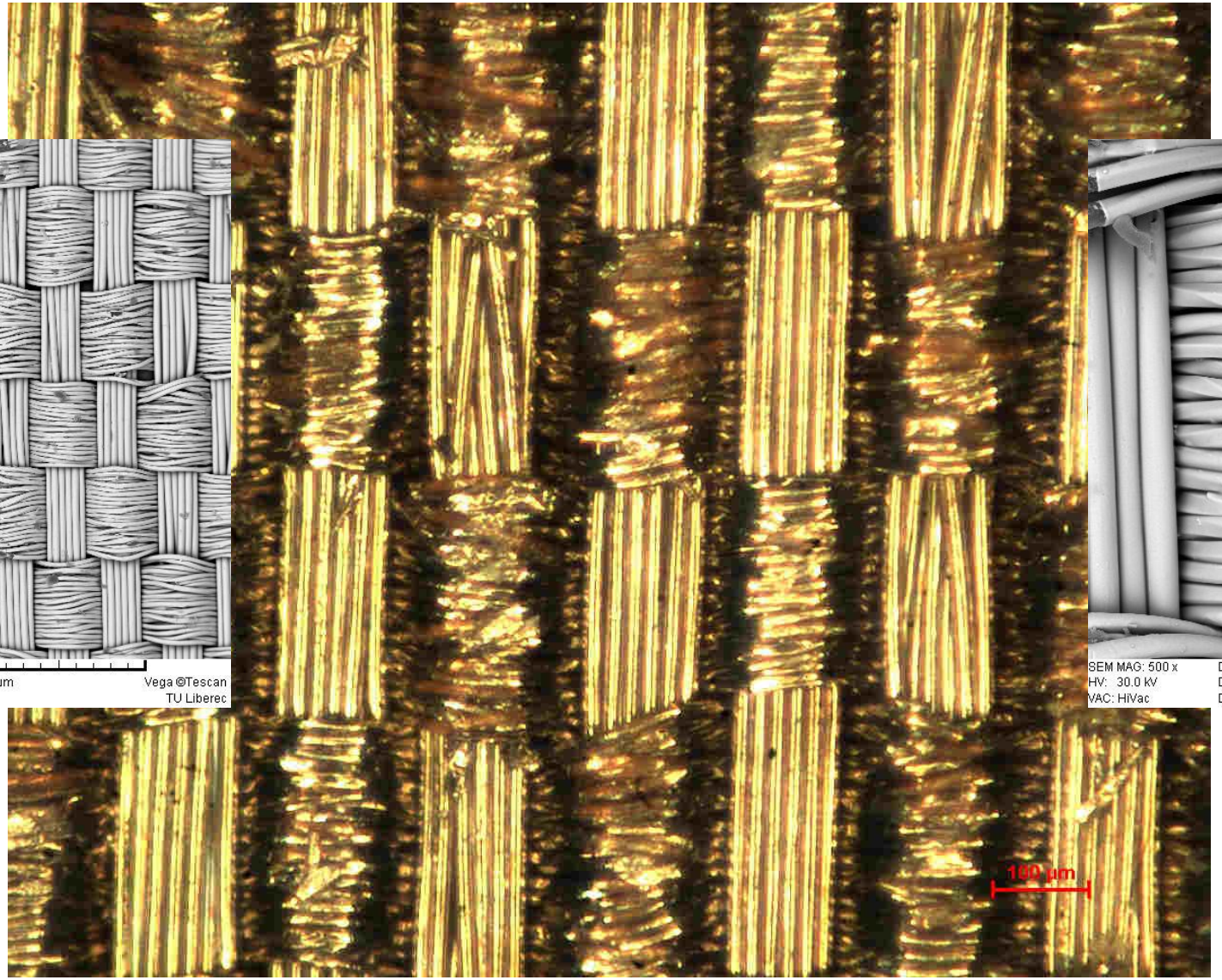
Examples of fabric images (sections, longitudinal views) on solved problems

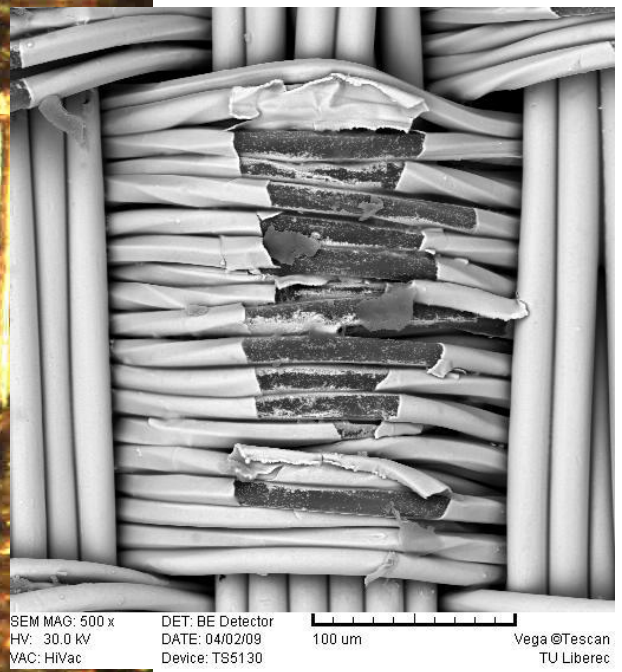
Hair authentication



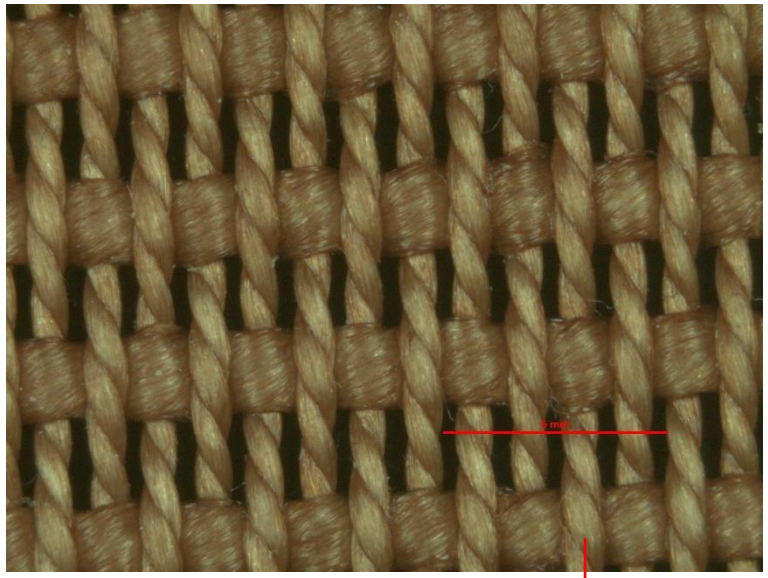
Stain analysis on technical fabric

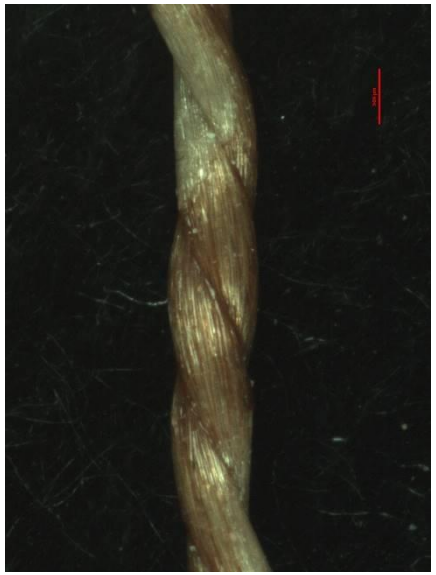


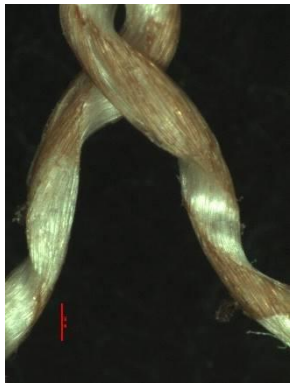


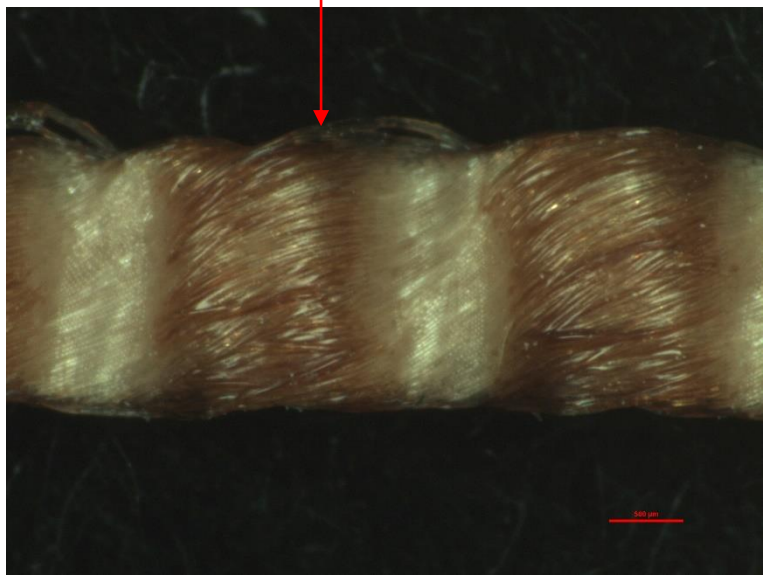


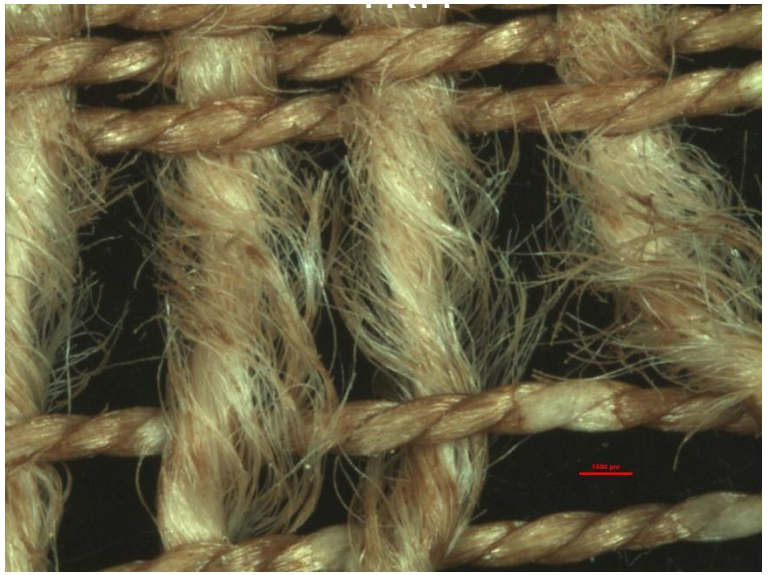
Analysis of technical textiles



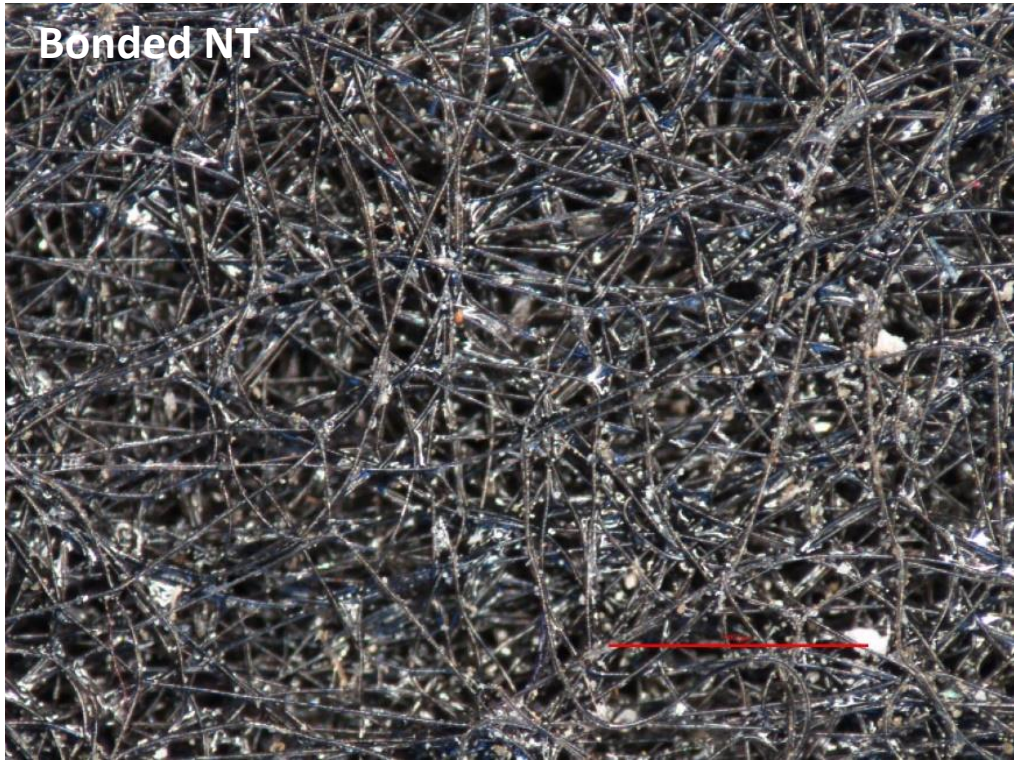


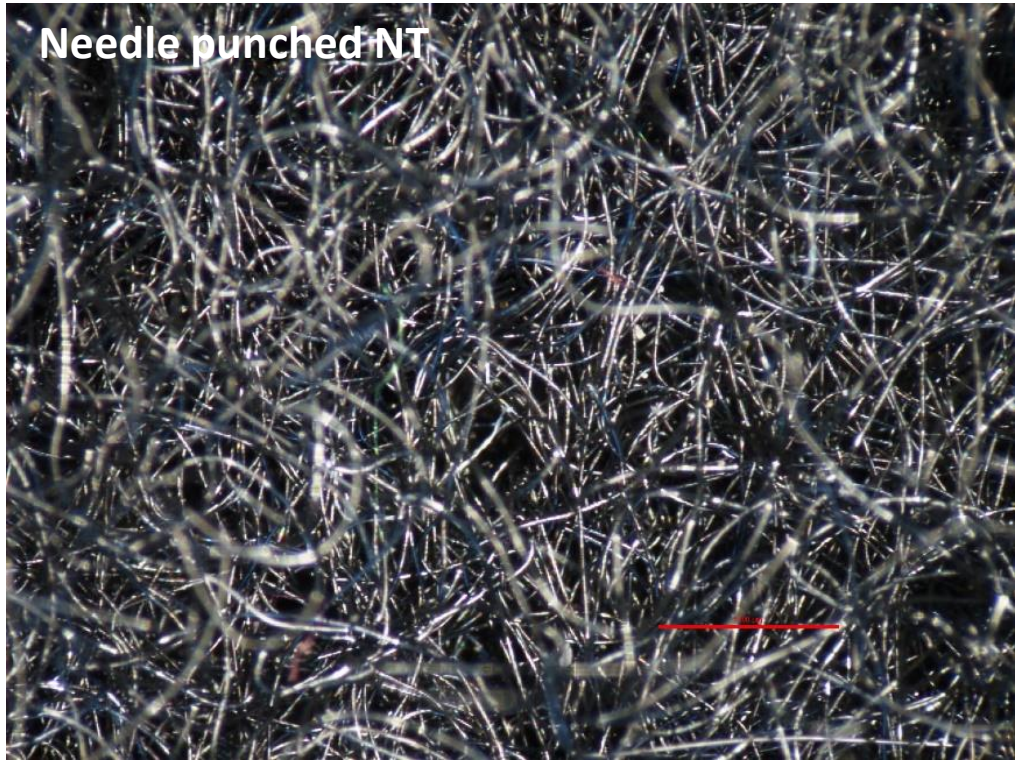




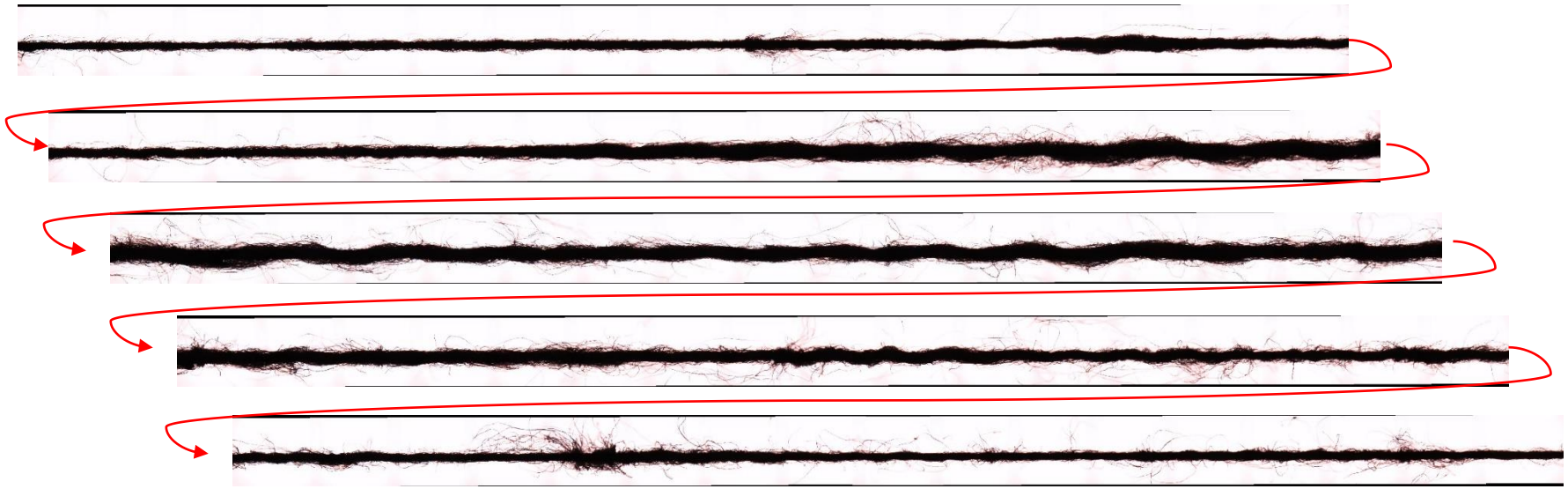


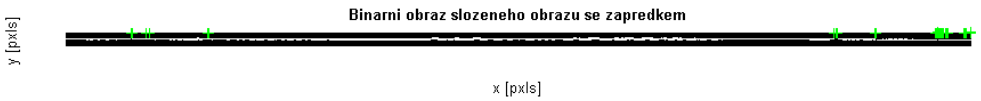
Determination of the production technology of two nonwovens

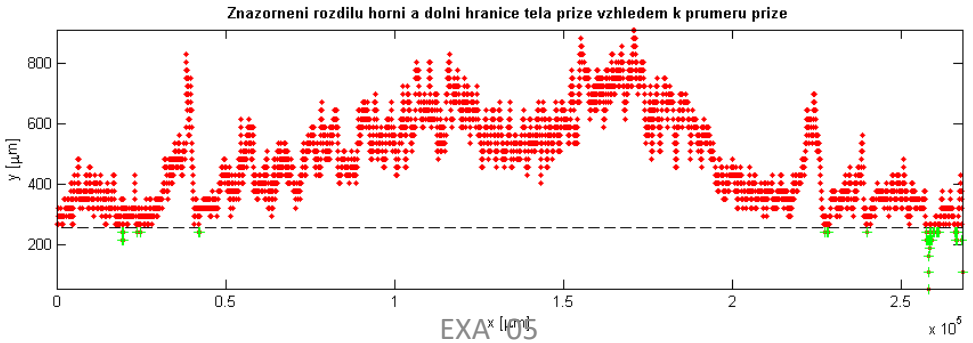




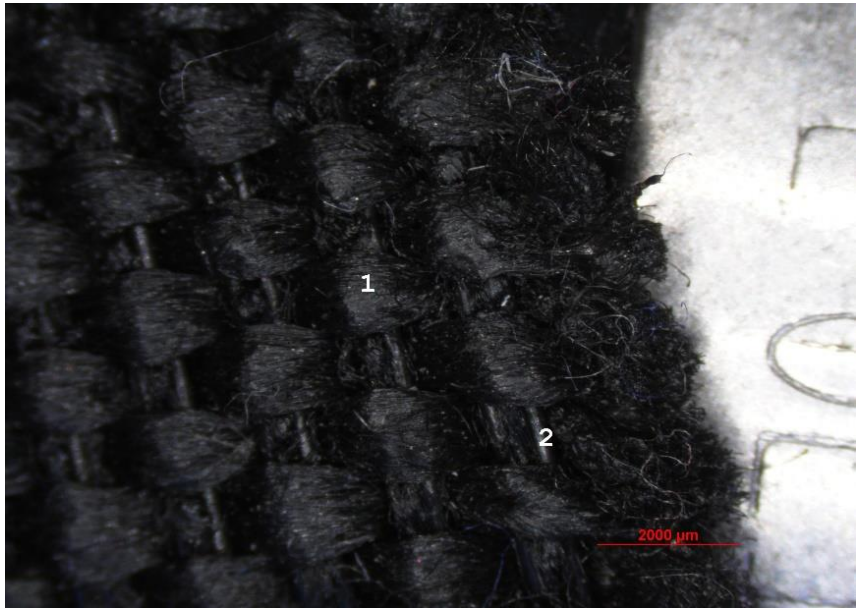
Evaluation of spinneret parameters

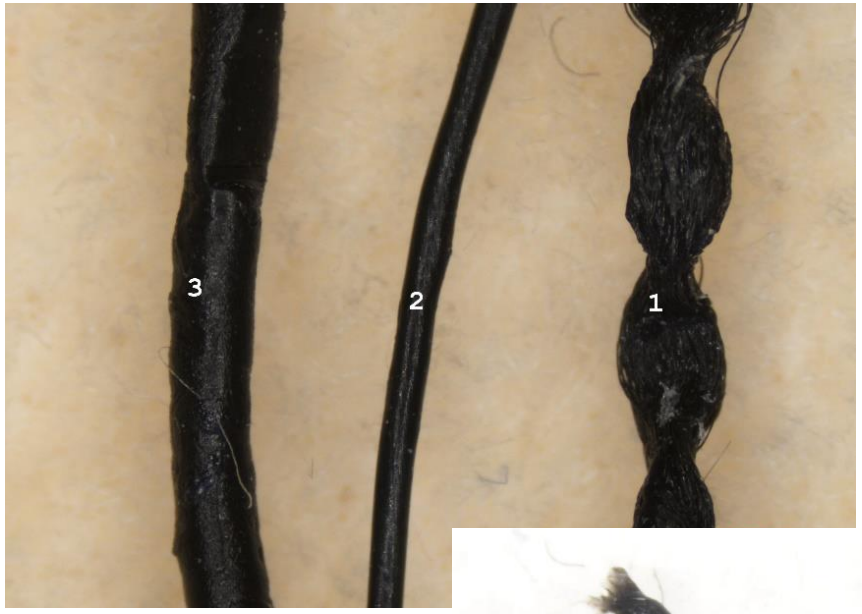




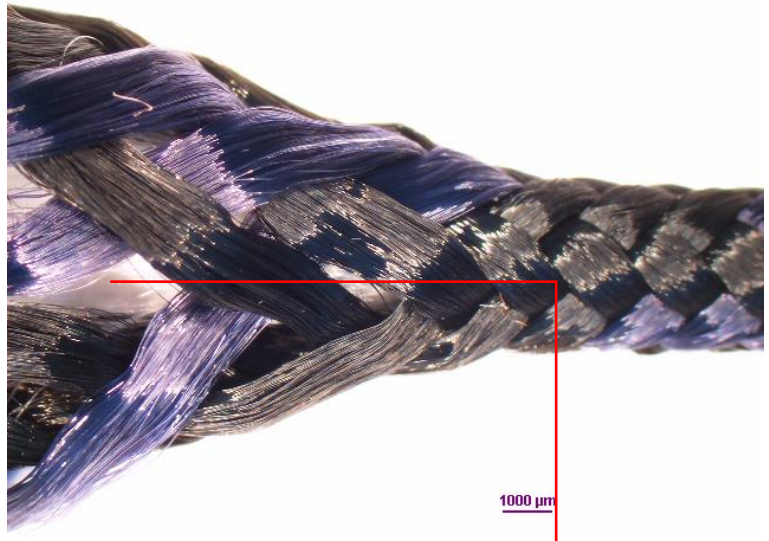


Analysis of technical textiles

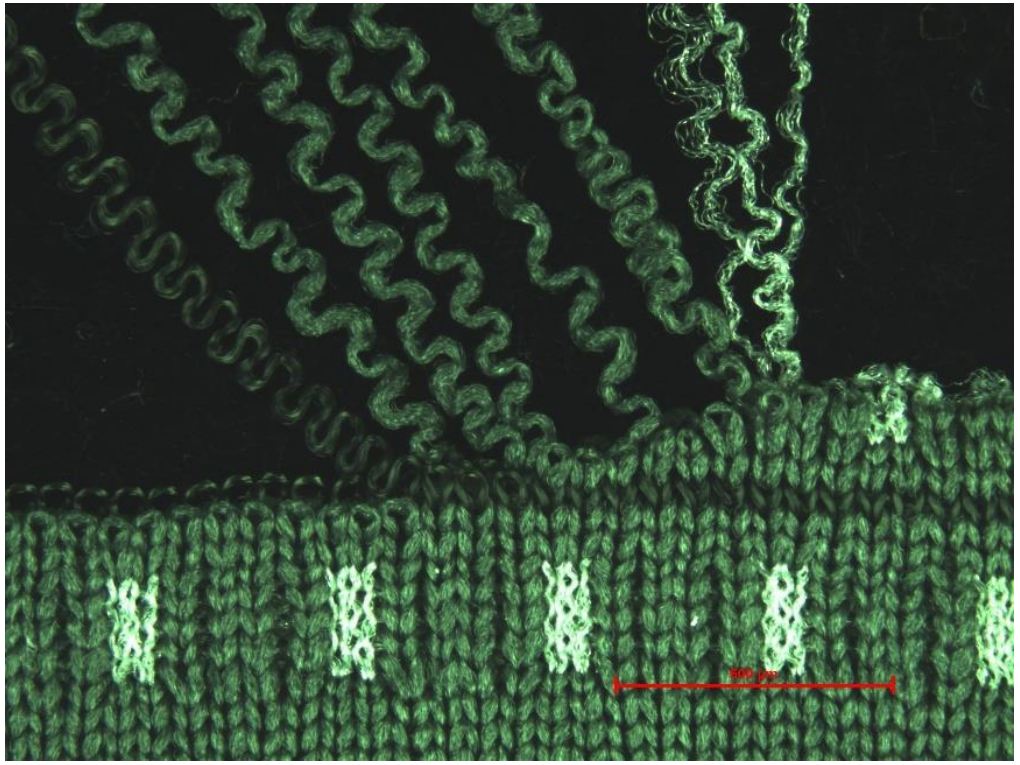


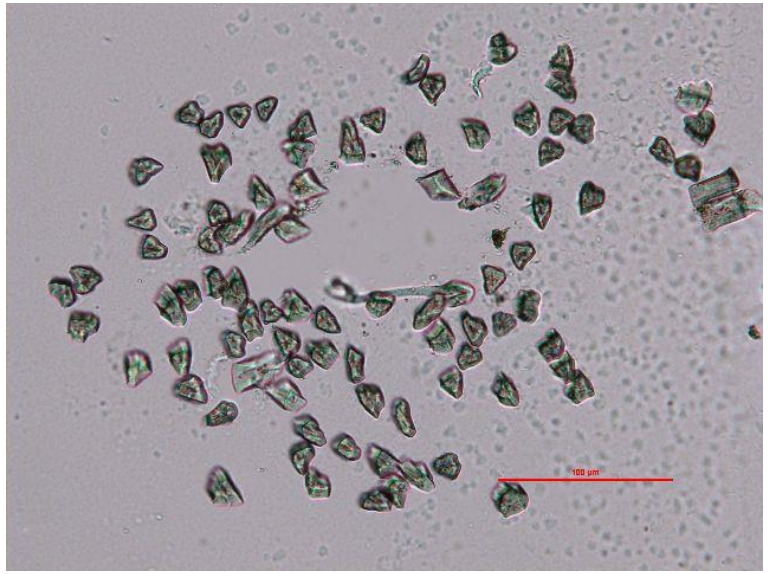


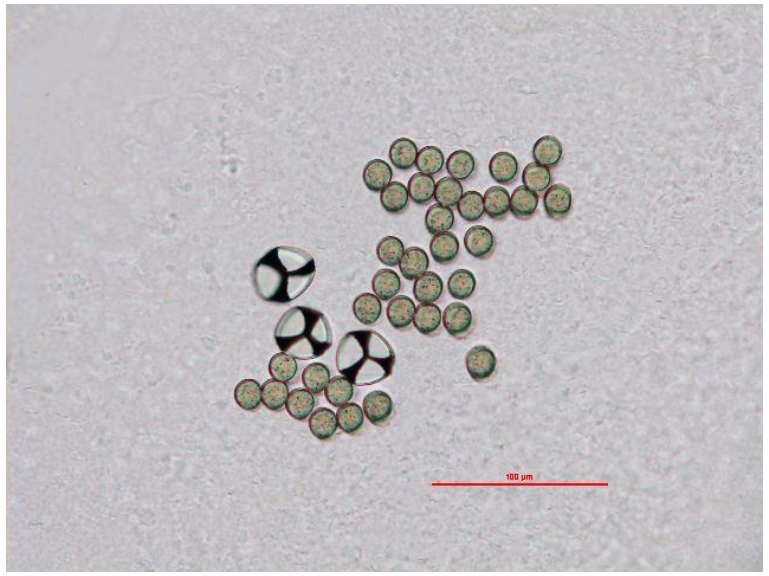
Analysis of the structure of braided rope with braided core



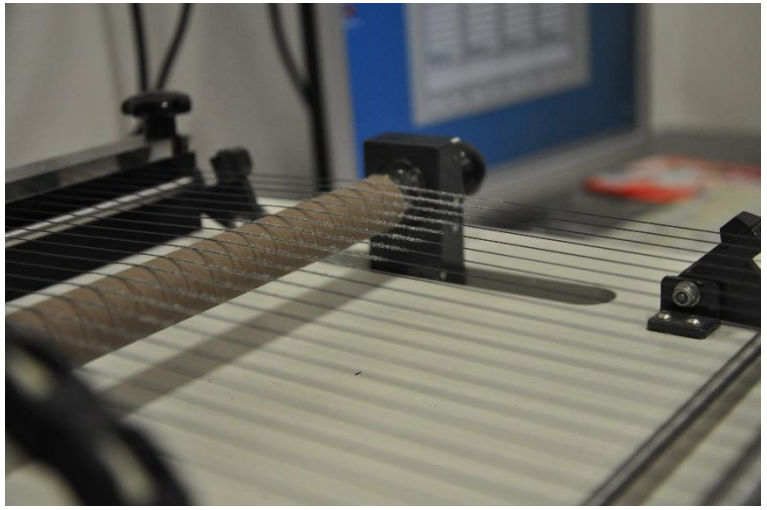
Analysis of the structure of a single jersey knitted fabric

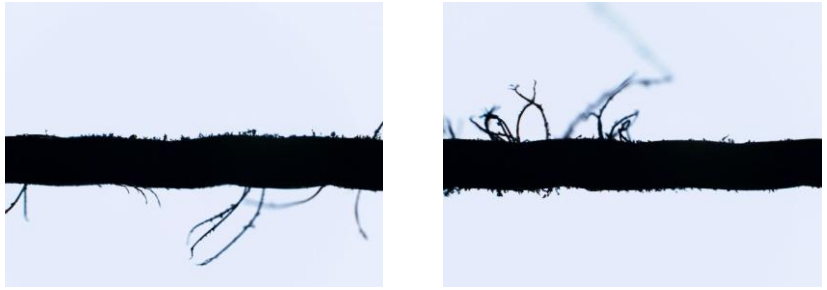






Monitoring damage to two ply yarn during abrasion

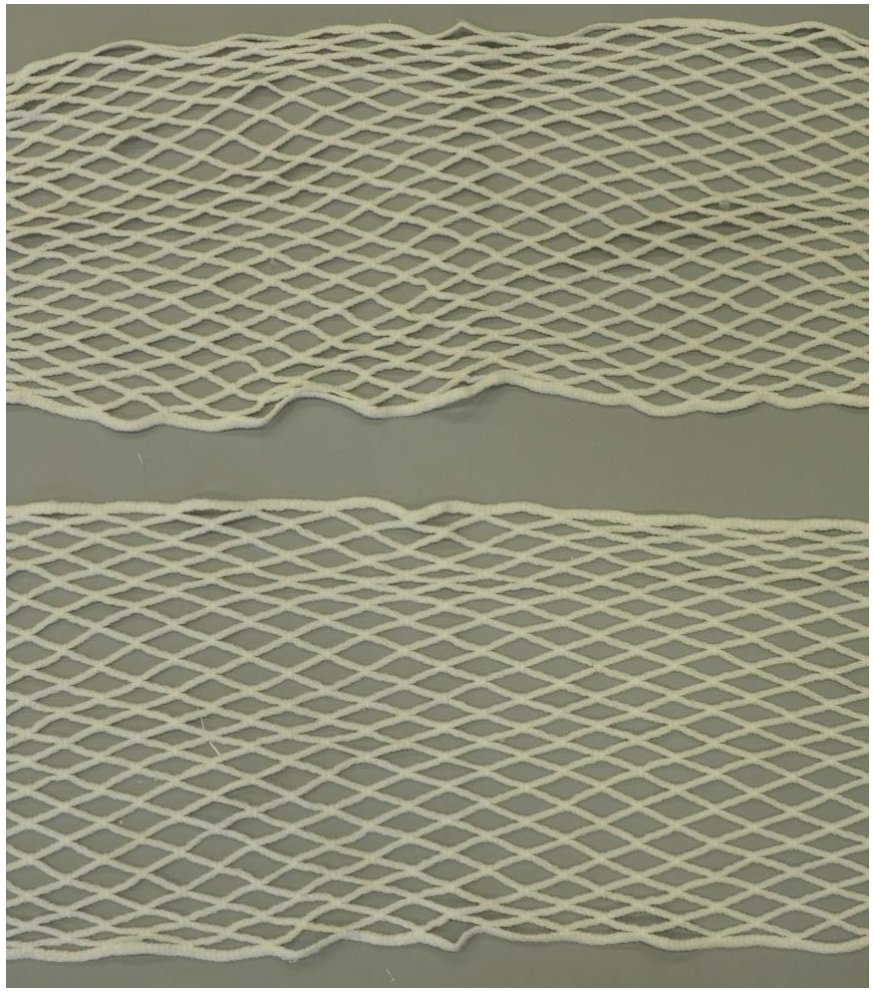


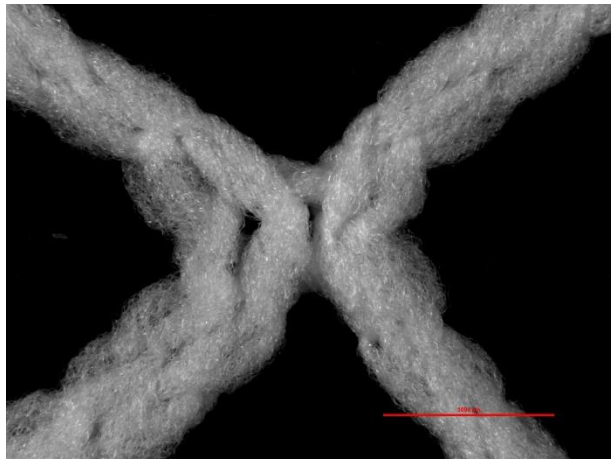


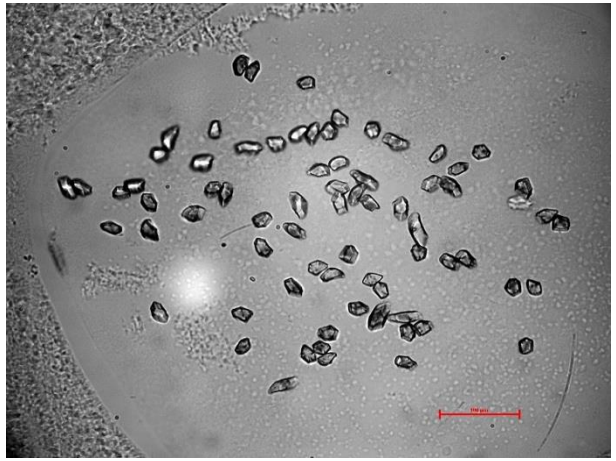
- after 250 abrasion cycles

- after 500 abrasion cycles

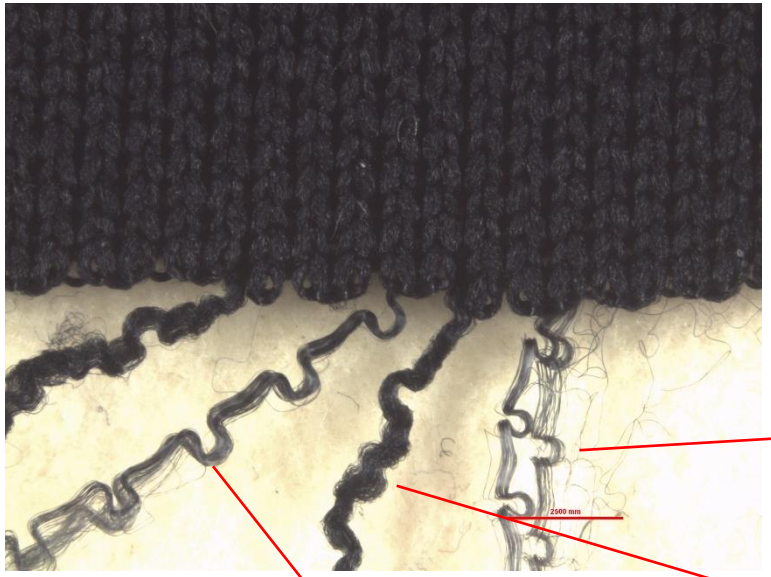
Analysis of the structure of the warp knitted fabric

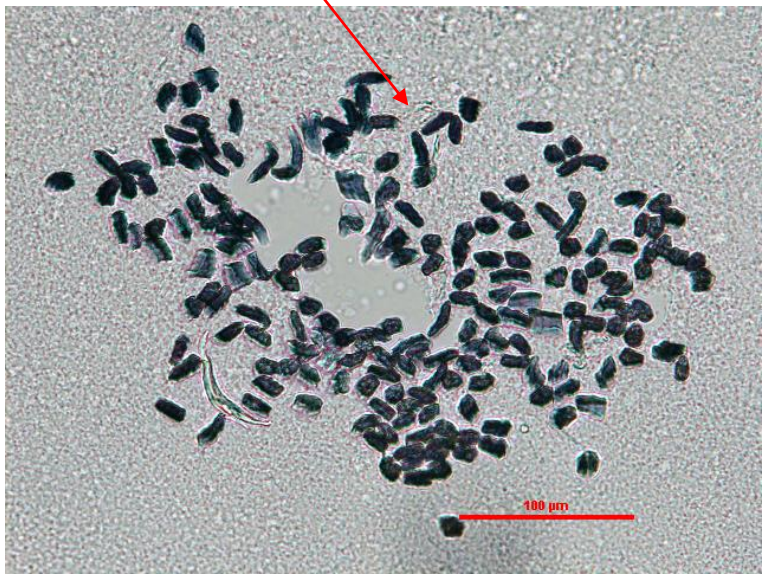






Does the submitted knitted fabric contain hollow fibres?





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