Project SESKUPIT

Masaryk University, Faculty of Science

Manual for laboratory courses:

Methods of sample decomposition: cryogenic grinding microwave decomposition

Solution analysis: ICP OES and ICP MS

Laser Ablation based methods: LA-ICP-MS

Laser-Induced Breakdown Spectroscopy LIBS

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2017

Methods of sample decomposition: cryogenic grinding microwave decomposition

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Determination of copper in a blue print by ICP-OES

Nowadays, analytical chemistry encounters the necessity of quantitative determination of elements in samples of all kind of states. Liquid and gas samples can be easily carried into flame or analytical plasma such as widely used inductively coupled plasma (ICP). If there is no possibility, or we just don't want to bring samples directly into excitation source by spark or laser ablation, it is necessary to transform solid and suspense samples into liquid solution. One of the common ways is to dissolve the sample in acid solution or in a mixture of acids with addition of other reagents. However, the correct procedure depends on the type of sample. Visible dissolution of sample does not necessarily mean that the sample was quantitatively dissolved or that there wasn't loss of one of the sample's component. Efficiency and the speed of the decomposition can be increased for instance by increasing temperature using a heating source or even better by microwave decomposition system. Furthermore, it's much easier to dissolve powder sample with small particles, than monoliths such as stones or metals. For this purpose, there is more than a one method; most common are blending or grinding. Grinding and homogenisation of sample are often necessary steps in sample preparation of tablets before X-ray analysis or laser ablation with ICP.

This task concerns the use of modern methods of a microwave decomposition and a cryogenic grinding for a solid sample preparation and analysis of this sample using ICP. A paper with a blue colour ink from basic PC printer is a good sample to demonstrate microwave decomposition as well as cryogenic grinding for copper determination in this ink.

Cryogenic grinding for sample preparation

Cryogenic grinding is based on cooling samples to cryogenic temperatures using liquid nitrogen, then pulverizes them by magnetically shuttling a steel impactor back and forth against two stationary end-plugs in a closed vial. This method makes it possible to grind even materials sensitive to heat or materials that are impossible to grind at normal temperature such as thermoplastics, hairs, textile or organic tissues. Cryogenic grinding is mostly suitable for materials that are soft and flexible at room temperature. Extremely low temperatures during the grinding suppress recrystallization and leads to finer grain structures.

Major advantages of cryogenic grinding are higher yield of fine particles in target range and uniform particle size distribution.

Fig. 1 Cryogenic mill 6775 Freezer/Mill

Procedure:

1.) Sample preparation

Blue square is printed on paper by an ink printer. Area of the printed colour is 10x10 cm. This sample is then cut into small squares ca. 1x1 cm and put into grinding vial (Fig. 2) with a steel impactor.

Along with the sample it is necessary to prepare a blank sample; in this case it is pure unprinted paper. Given the fact, that grinding takes about 25 minutes, it is best to prepare blank sample during the sample grinding.

Fig. 2 Grinding vials

2.) Grinding

Vial with the sample is placed into the mill in the vial slot. It is essential to check the amount of liquid nitrogen in the container (the level of nitrogen cannot drop more than 2 cm below the top edge of the container). Afterwards, the container is closed and secured with rubber clip. Programme for paper grinding is started from the screen.

Parameters for paper grinding are shown in table 1.

parameter	value
Precooling	15 min
Grinding	1,5 min
Cooling	2 min
Nr. of cycles	3
rate	12 cps

Tab. 1 Grinding parameters

3.) Sample removal

When the programme finishes, the freezer mill is opened and the vial is removed using magnetic pen. Be careful when handling the vial, its metal parts are frozen to cryogenic temperature. Then the vial is opened using opener (Fig. 3) by removing the plug. Grinded sample is then spilled on filter paper and placed into exicator.

Pic. 3 Grinding vial opener

4.) Microwave decomposition

Microwave decomposition is commonly used for sample dissolution prior to elemental analysis using methods ICP-MS, ICP-OES or AAS. Advantages of microwave decomposition are shortening of the time of the analysis, reducing of the amount of acid needed, reducing of the risk of contamination or decomposition of samples, that are difficult to decompose. Microwave decomposition can be either in open system (at atmospheric pressure) or closed system. In open systems, the boiling point of the acid establishes

Fig. 4 Microwave system ETHOS ONE

the maximum decomposition temperature, whereas in closed systems is maximum limited to 250°C.

Usually, different mineral acids are used for sample decomposition (e.g. HCl, HNO_3, HF, H_2SO_4...) or hydrogen peroxide. Vials for decomposition are made of inert material (such as teflon) which are transparent for microwave radiation. Heating by microwave radiation depends on type of the sample, therefore it is necessary to monitor temperature of samples. Decomposition will be carried out in microwave decomposition system ETHOS ONE (Fig. 4).

Principles of working with microwave decomposition system

- Vials must be clean and dry, therefore it is necessary to use gloves when operating.
- Vials and their caps are numbered, it is necessary to keep sets together.
- Output from microwave system leads to hood, which must be always on.
- Vial number 1, reference vial with thermal sensor, must always be at position 1. In this vial, temperature is measured, therefore it is crucial to use it for a sample, not a blank.

Reagents
6ml HNO_3 (65%), 2ml H_2O_2 (30%)

Programme

Step	Time	Energy	T1	T2
1	00:15:00		200	ramp time
2	00:15:00		200	decomposition
3	00:10:00		-	cooling

Tab. 2 Parameters for temperature programme

Procedure:

1.) Sample preparation

0,1 g of sample is put into decomposition vial and using acid. (Fig. 5). Analogously, blank sample is put into second vial. Both vials are put into safety cover and then the whole segment is closed (Fig. 6.). Using torque wrench the pressure gauge is tightened until it clicks. Now the segments are ready to be put into microwave system. It is necessary to put there at least 3 vials, so the system is balanced. Therefore, we use same amount of acids in the 3rd vial. All 3 segments are evenly embedded into microwave system.

Fig. 5 Vial for microwave decomposition

Tlakový závit
Bezpečnostní pružina
Disk
Indikační kroužek
Nádobka
Ochranný kryt
Tělo rotoru

Fig. 6 Segment for decomposition of one sample
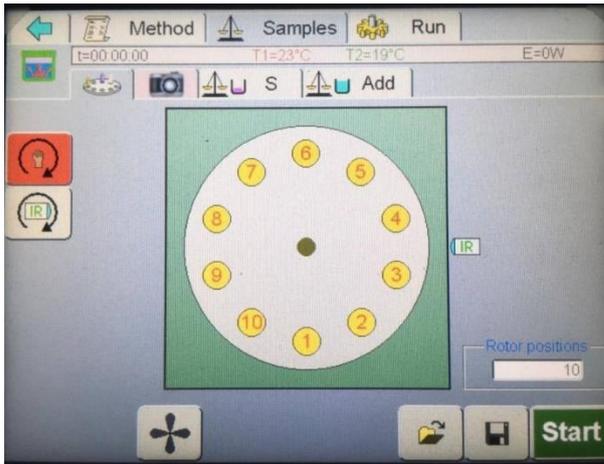
Choosing a position on *Samples* touch screen (Fig. 7) will rotate the disk so that the chosen position is in the front. Reference vial is placed in the position 1 as the last one. After placing all segments except the reference one, an upper disc is placed on top of them as shown on Fig. 8. Then the reference segment is placed and the thermal sensor is connected via cable. Lastly, the upper disc is fitted onto all segments so that all segments are fixed.

Fig. 7 Sample screen

Output of thermal sensor

Upper disc

Segment with sample

Bottom disc with positions
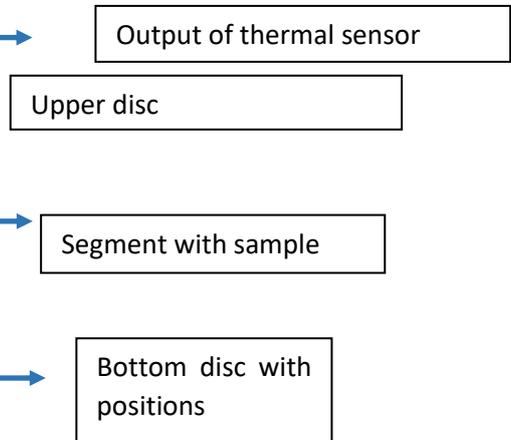
Fig. 8 Segments with upper disc

2.) Programme settings

It is important to check all boxes on the home screen exactly as is shown on Fig. 9. Be sure to check the TWIST button, without it the cable of thermal sensor would be torn up. It is possible to check the correct rotation of the rotor using button with a fan. (Fig. 10).

Fig. 10 Rotation control button
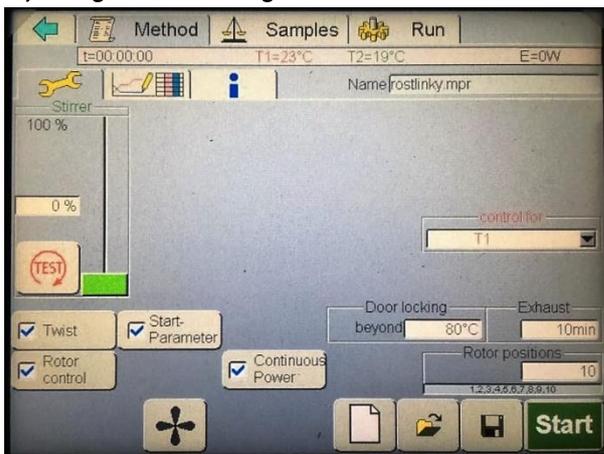
Fig. 9 Settings home screen
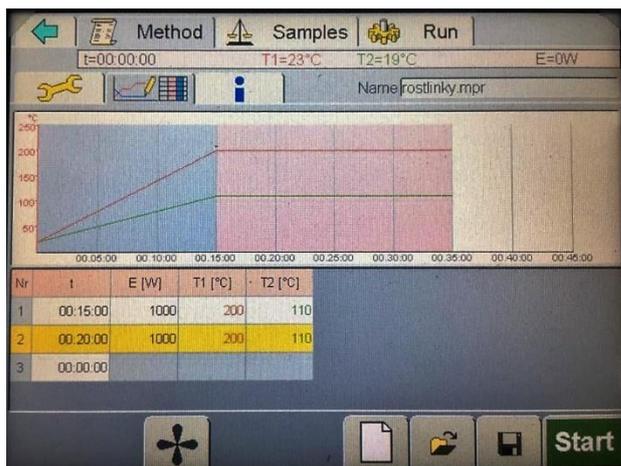
Fig. 11 Method tab

Setting of the thermal programme is done in the *Method tab*. Table is filled with parameters for microwave decomposition programme. Step 1 is the time of the gradient increase of the temperature until the required temperature is reached. Step 2 is the time of stable temperature. The graph above the table shows temperature profile of the programme. Using save button is the method saved. If the method was already created, it is possible to load it from saved files using load button.
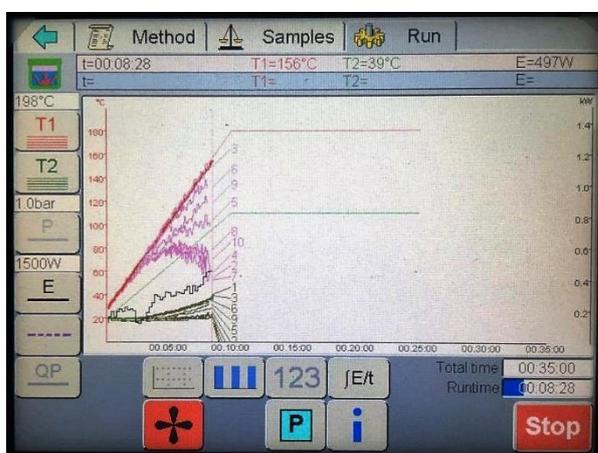
Fig. 12 Run tab

To launch the programme the *Start button (in the Run tab) is pushed*. The progress of the decomposition is shown on the Run screen. (pic. 12). The door is automatically locked at 80 °C. after the decomposition it is necessary to wait until the temperature of the samples drops to about 50 °C. After that it is possible to remove them from microwave and open them using torque wrench. Samples are then quantitatively transferred into 25 ml volumetric flasks.

Inductively coupled plasma optical emission spectrometry (ICP-OES)

ICP-OES is an analytical method based on the determination of the intensity of emitted light from excited atoms and ions. Sample is introduced into the ICP as liquid form using nebuliser. Inside of ICP, aerosol is evaporated and dried. The plasma excites atoms, which leads to photon emission and ionization. Deexcitation causes radiation at the characteristic wavelengths of the elements involved, and its intensity is measured with detector. Emission spectra of atoms an ions offers qualitative information (wavelength) as well as quantitative information (intensity).

- **Atomisation and excitation source**

  The excitation source must desolvate, atomize, and excite the analyte atoms. As atomisation and excitation source in ICP-OES is used electrical discharge, that creates inductively coupled plasma. An inductively coupled plasma can be generated by directing the energy of a radio frequency generator into a suitable gas, usually ICP argon. This plasma has high electron density and temperature (10000K) and its energy is used in the excitation-emission of the atoms from the sample.

- **Optics of spectral instrument**

   Electromagnetic radiation is emitted during deexcitation of particles inside of the plasma. This radiation consists of analytically important radiation of atoms and ions of the sample and radiation of non-dissociated molecules (OH, CO, N_2...), which does not carry analytical information and therefore creates noise. For analytical purposes, it is necessary to split this polychromatic radiation to individual components (emission lines), which are characteristic for elements. For this purpose, a dispersive element is used, such as prisms, gratings or filters. This component is one of the most important part of an ICP optical spectrometer, since it is their quality that has the major impact on resolution. Prisms and filters don't achieve resolution needed for ICP-OES and therefore only gratings are used. Based on the type of grating, ICP-OES spectrometers can be divided into 3 groups:

   - *spectrometer with planar diffraction grating Czerny-Turner*
   - *spectrometer with concave diffraction grating Paschen-Runge*
   - *spectrometer with echelle grating*

Deretmination of Cu in the sample will be carried out using ICP spectrometer iCAP 6500 Duo (Thermo Scientific). ICP spectrometer contains echelle monochromator with grating with 52,9 grooves per mm. This system offers simultaneous measuring of spectrum in the range 166 –847 nm.

Fig. 14 ICP Spectrometer ICAP 6000 series

Procedure:

1.) *Sample and calibration standards preparation*

   Calibration standards with concentration of Cu^{2+} 1 mg/l, 0,5 mg/l a 0,1 mg/l a 0 mg/l is prepared from calibration standard Astasol (c_{Cu} = 1 g/l). It is important to add the same amount of acid to each calibration solution as was added to sample. Samples don't need to be adjusted anymore.

2.) Method settings
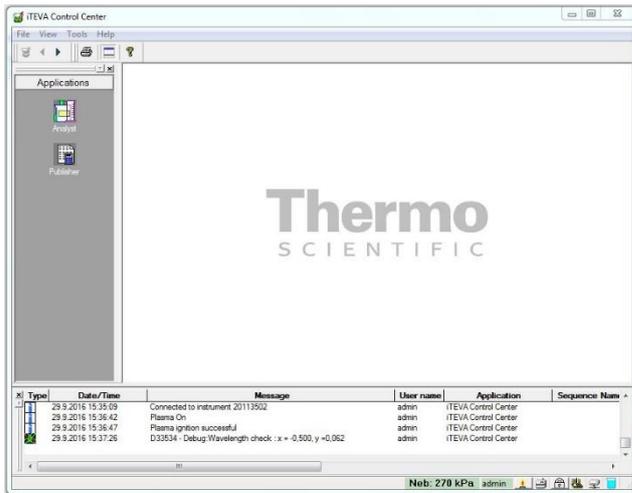
Fig. 15 Home screen of iTEVA

Spectrometer is controlled using programme iTEVA (Pic. 15). At the bottom bar choose lock icon, which opens „Interlocks" window, where actual state of spectrometer is shown (communication with PC, exhaust system,…) including actual temperature of detector (Fig. 16). If any of Interlocks is red and the temperature of the detector is -45°C, it is possible to start plasma.

Fig. 16 Instrument status
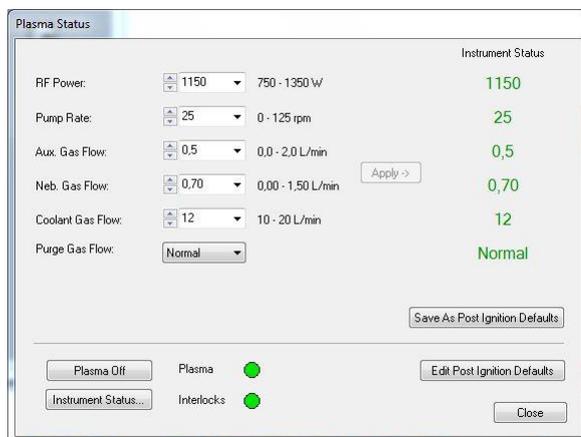
Fig. 17 Plasma status

Icon of the burner will open window with the table „Plasma status" (Fig. 17), where the button Plasma On is chosen. On the home screen of iTEVA choose the tab Analyst. New method is created as follows:

Method => New
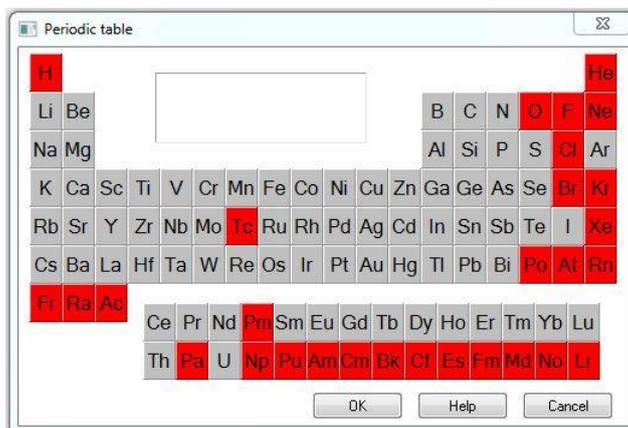
Fig. 18 Periodic table for the choice of elements

Fig. 12 Selection of Cu lines

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Window with a periodic table is opened (Fig. 18.). Measured lines of Cu are selected after the selection of measured element – Cu. (pic. 19.). Although, individual lines are sorted based on their intensity, it is necessary to control interferences (right part).
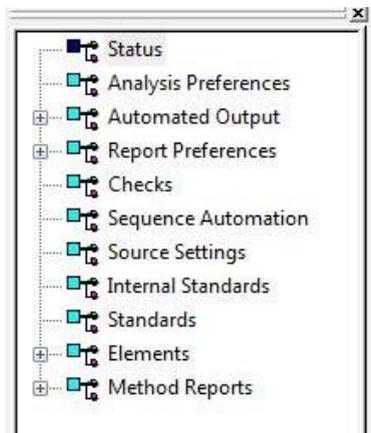
Fig. 20 Method settings tabs
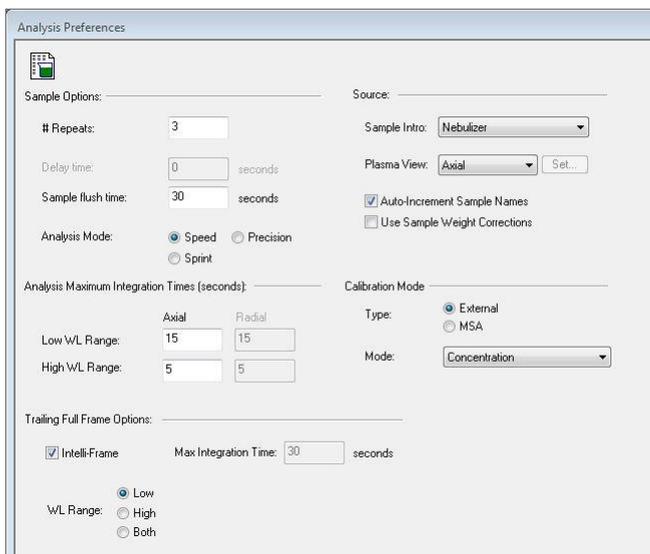
Fig. 21 Analysis preferences

From method settings tabs (Fig. 20.) choose *Analysis Preferences* (Fig. 21.) and select axial view, which is often used for determination of elements with low content in samples with simple matrix. Axially viewed plasma system looks down the central channel of the plasma and collects all the analyte emission over the entire length of the plasma. In radial plasma, spectrometer views the analyte emission from the side of the plasma through the background argon emission.
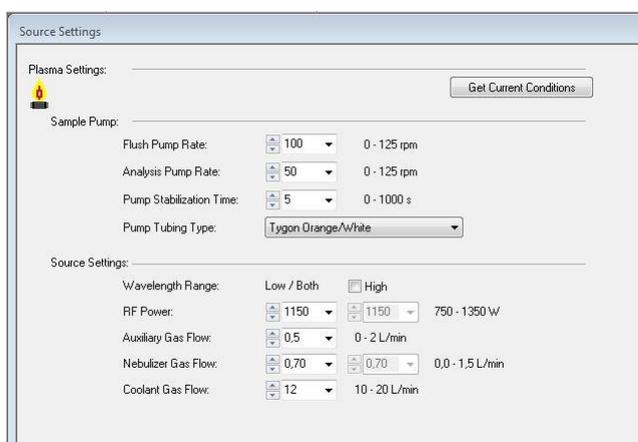
Fig. 22 Source settings

In *Source Settings (Fig. 22.)* set up following parameters:

Parameter	Value
Flush pump rate	75 rpm
Analysis pump rate	25 rpm
Pump stabilisation time	5 s
Nebuliser gas flow	0,65 L/min

Tab. 3 Parameters for measurement
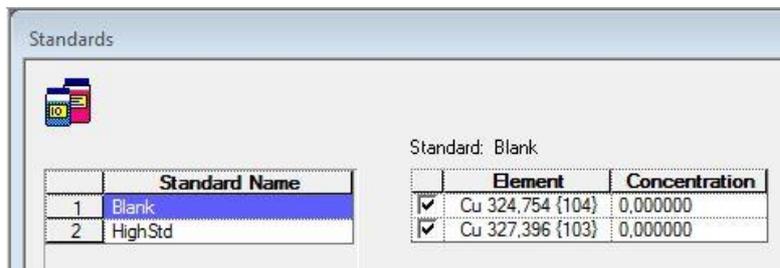
Fig. 23 Standards settings

For each standard, set its concentration in *Standards window* (Fig. 23.). If needed, using *Add button* it is possible to add more standards. As next step save the method and choose *Analyst tab*.

3.) Calibration and analysis

Fig. 24 Upper bar with icons
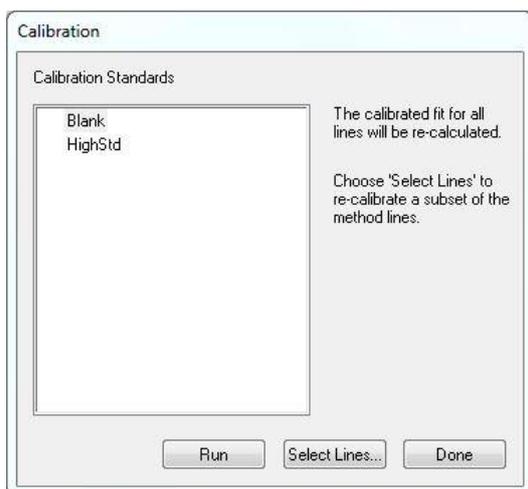
Fig. 25 Calibration window

Open the calibration window (Fig. 25.) by pressinng a button with purple beaker (Fig. 24.) at the upper bar. Immerse the nebulizer sample tube into calibration blank and start the measurement by pressing *Run* button. Analogously measure the calibration solutions. After measuring all the calibration standards finish the measurement by pressing the button *Done*. Analysis of sample is started by pressing the green beaker (Fig. 24.). It is good to check stability when measuring large number of samples by measuring one of the standards at the end of the measurement again.

4.) Data export
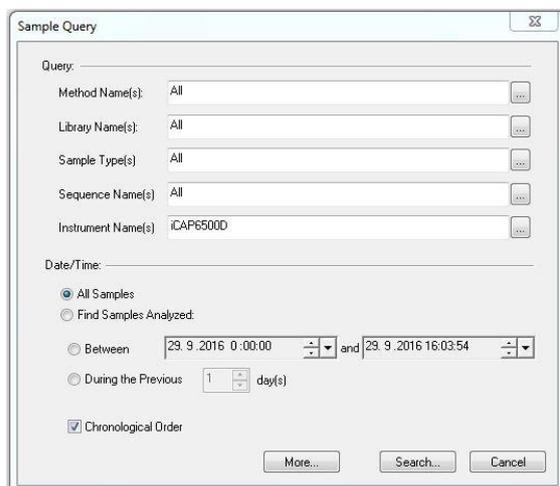
Pic. 26 Selection of samples
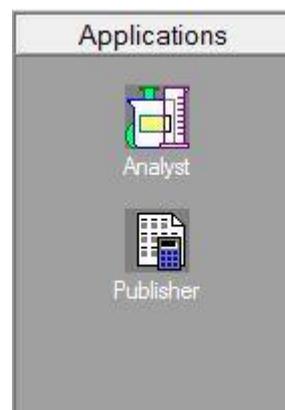
Pic. 27 Publisher icon

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Measured data are exported using the Publisher icon *(pic. 27.)*, right below the *Analyst* icon.

New report => Sample report => OK

In *Sample query* window (pic. 26.) set the date of measurement and select method used. Select analysed samples from the list for which data are to be exported. Select the sample and press *OK*.

Data file is exported as follows:

Export => Excel => Save

Data evaluation

The precision of the result is limited by the random errors. Random errors in experimental measurements are caused by unknown and unpredictable changes in the experiment. Random errors often have a Gaussian normal distribution. The mean *m* of measurements of the same quantity is the best estimate of that quantity, and the standard deviation *s* of the measurements shows the accuracy of the estimate. When number of measurements is small n << 10, instead of standard deviation is calculated from range R:

$$s_R = k_n \cdot R$$

Where k_n is Dean-Dixon coefficient (k_3 = 0,5908).

Confidence interval

Confidence intervals consist of a range of values (interval) that act as good estimates of the unknown value. Dean-Dixon tailored a method of the calculation of confidence interval for small sample size datasets:

$$L_{1,2} = \bar{x} \pm K_\alpha \cdot R$$

where K_α is critical value of Lord distribution for confidence level α.

(for α=0,05 k_3 = 1,304)

Limit of detection and limit of quantification

These limits are calculated from value $\bar{x}_B$ (mean of 10 measurements of blank) and the standard deviation $\bar{\sigma}_B$ (also calculated from 10 measurements of blank).

$$\text{LoD} = \bar{x}_B + 3\bar{\sigma}_B$$

$$\text{LoQ} = \bar{x}_B + 10\bar{\sigma}_B$$

Hypothesis testing

Two-sample test for a difference in mean is used when measuring sample by 2 different methods. When the number of measurements ($n_A = n_B = n$) is most often done by simplified Student t-test. For small size datasets is commonly used the Lord test:

$$u = \frac{|\bar{x}_A - \bar{x}_B|}{R_A + R_B}$$

If the value of *u* is greater than critical value of Lord test at the chosen significance level α, the difference in means is statistically significant. In that case the results from different measurements cannot be considered as identical (for α=0,05 u_α = 0,636)

Determination of copper in a blue print by ICP-MS

Principle of ICP – MS

Liquid sample is transformed to the aerosol by nebuliser and transported to the inductively coupled of plasma with its temperature between 6 000 and 10 000 K (depending on plasma region). Here is aerosol dried, evaporated, atomized and ionized. In ICP the ionization occurs into the first degree only, except alkali metals and rare earth elements. Ions created in the ICP source enter into mass spectrometer via interface system. The interface consists of sampler and skimmer cones and there is an effective separation of argon flow from atoms and ions flow occurred here. First the plasma beam (ions and atoms from the sample and carrier gas) go through the sampler cone into pre-vacuum space with pressure of 100 Pa, approximately. The supersonic expansion occurs with subsequent cooling of the plasma beam. Then it enters into ion optic via skimmer. The ion optic serves to focusing of the beam and removing of photons and non-charged atoms that would increase the background level of the detector. Focused ion beam enters into collision-reaction cell filled with He that suppress possible polyatomic interferences. Then the ions are separated in the quadrupole analyser according to their mass to charge ratio (m/z) and ions with selected m/z exit from the analyser and hit the electron multiplier that generates signal (Fig. 1). For more details of ICP see instruction LA-ICP-MS.

Fig. 1: Schema of ICP-MS. (http://www.chem-agilent.com/contents.php?id=53926)
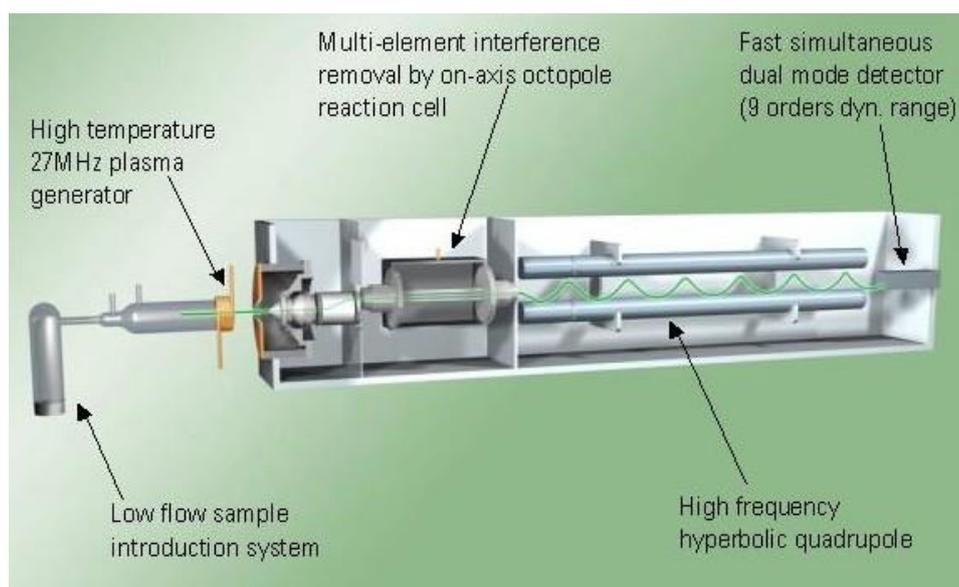
The aim of this practicum is:

a) to quantitative deremine the total content of copper in ink (blue color)
b) to prepare calibration standards for determination of copper
c) to learn how to measure on ICP-MS
d) to calculate the limit of detection (LOD)
e) to compare the LOD with another solution analysis method ICP-OES.

➢ Turning on the spectrometer:

Turn the argon (Fig. 2a) valves on the wall so that the machines have access to a flow of the gases. Turn on the cooling system (Fig. 2b). Now open the valve on the helium cylinder (Fig. 2c). Finally, turn on the computer.
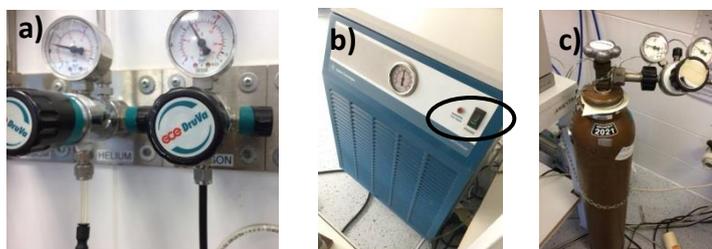
Fig. 2: Turning on of LA-ICP-MS, a) argon b) cooling, c) helium into collision cell.

First of all, set up measurement of solution analysis → open the program *Configuration*, → *Sample introduction - Type* click *Peristaltic Pump* (Fig. 3a). Now click *Miscellaneous* (Fig. 3b) → *SC Cooling* (Fig. 3c) → OK (Fig. 3d) → OK. Then open in PC program *ICP-MS-Top*.
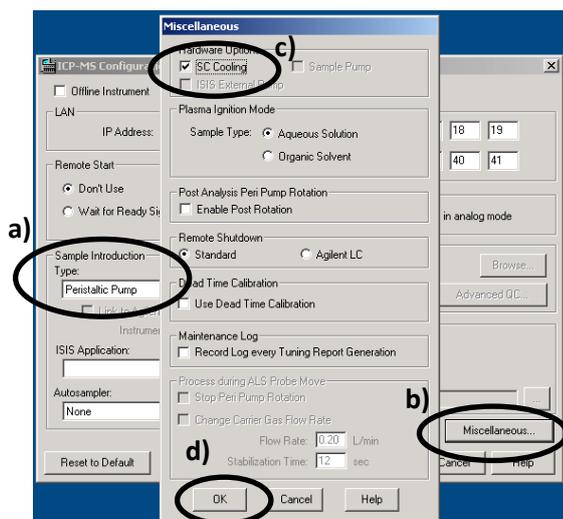
Fig. 3: ICP-MS configuration.
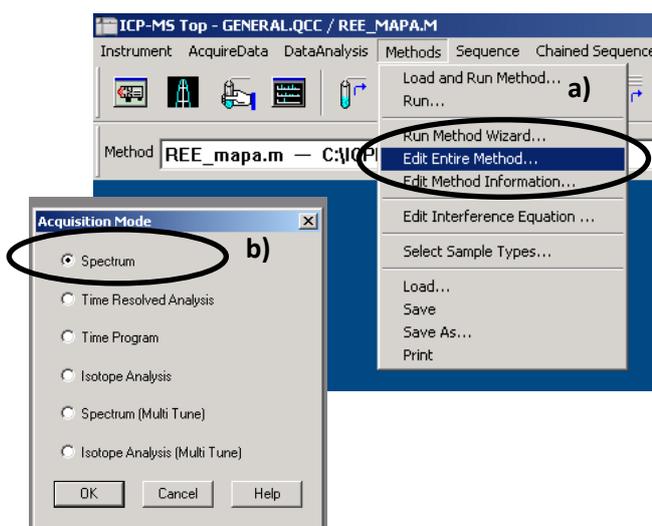
Fig. 4: Set up of method for ICP-MS.

➢ Analysis process:

a) Ignite the plasma

In order to start ICP move to the ICP-MS-Top program and click on the *plasma on* icon (Fig. 5). Wait for 45 minutes after the plasma is ignited to start measurements. Use this icon and set up the program for solution analysis → *file → Load Tuning Parameter File → He_roz.U → OK.*
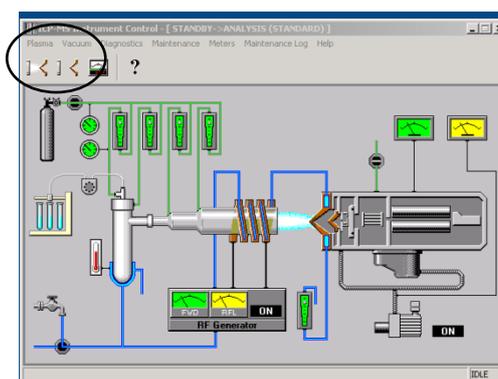
Fig. 5: Ignite the plasma.

b) *Set up the method of analysis*

In folder *data* and *methods* create own folder with analysis date. Next set up the parameters of method analysis **ICP-MS-Top:** Click to upper icon *Methods → Edit entire methods* (Fig. 4a) → select *Method information a Aquisition → ok →* create a description of the analysis being performed *(copper amount determination in ink) → Acquisition mode – spectrum* (Fig. 4b) → *ok → periodic table* (Fig. 7a) select ^{63}Cu and ^{66}Zn – internal standard (Fig. 7b) → *integration time* (Fig. 7c) ^{63}Cu fill *0.1 s* → OK → *Peristaltic pump* → Uptake Speed: *0.50* rps, Uptake Time: *40* sec, Stabilization Time: *10* sec.

Fig. 6: Program of peristaltic pump.

Now click *DataAnalysis* (Fig. 7d) → *Main panel* → now is opening the program **Online ICP-MS Data Analysis.**
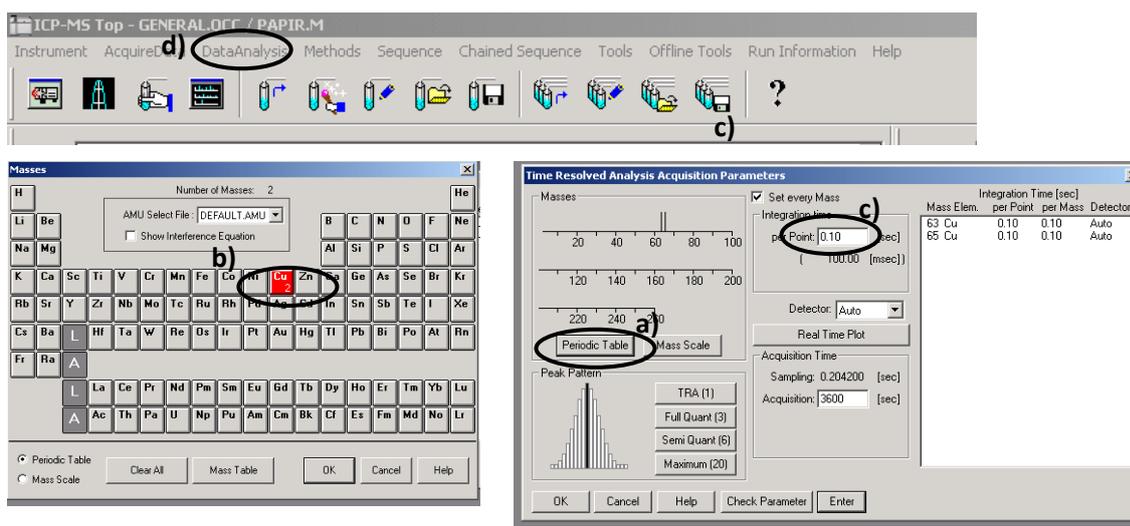
Fig. 7: Setting the acquisition method

c) *ICP-MS Data Analysis*

Once the data analysis program opens select the folder icon with a plus mark (Fig. 8a, here will be saved the results) → click data → name the folder → create (Fig. 8b).

Now go to the *DA Method* (Fig. 8c) → *c*lick *edit → Analys. Mode. → select Spectrum* (Fig. 8d). Then click the *green arrow icon*. (Fig. 8e) → *Load List* with element of interest → *Methods* and select the name of the folder → now you see the elements of interests → click the *green arrow icon* again (Fig. 8e)→ set up the units (ppb) and concentration of standards *(0, 100, 500, 1000* Fig. 9a) → *Return to Batch-at-a-Glance* (Fig. 9b) → *yes.*
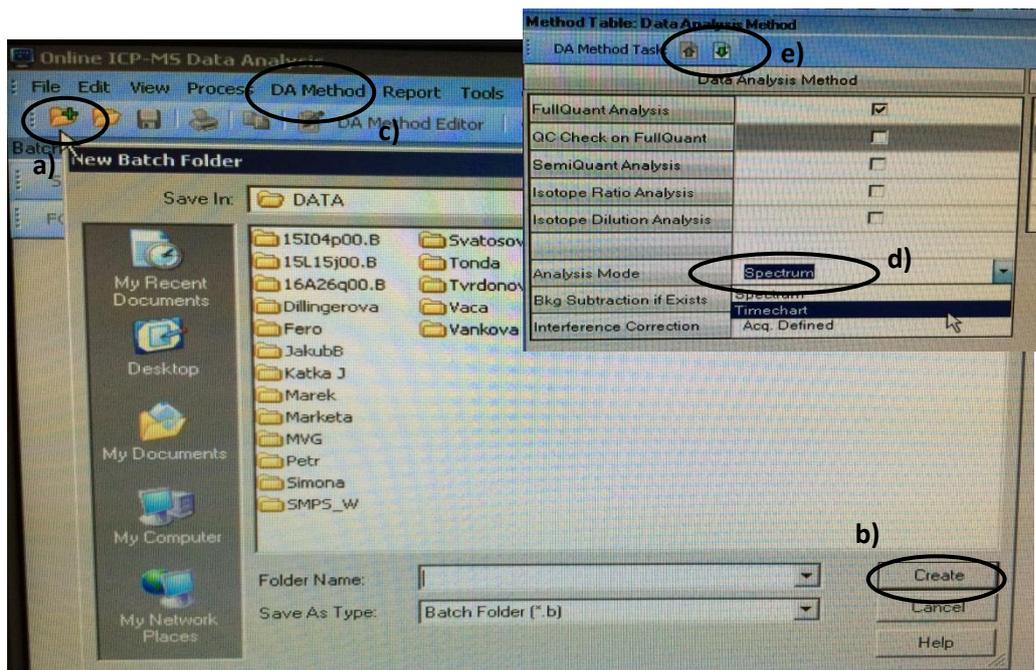
Fig. 8: Setting the measurement parameters in ICP-MS Data Analysis.
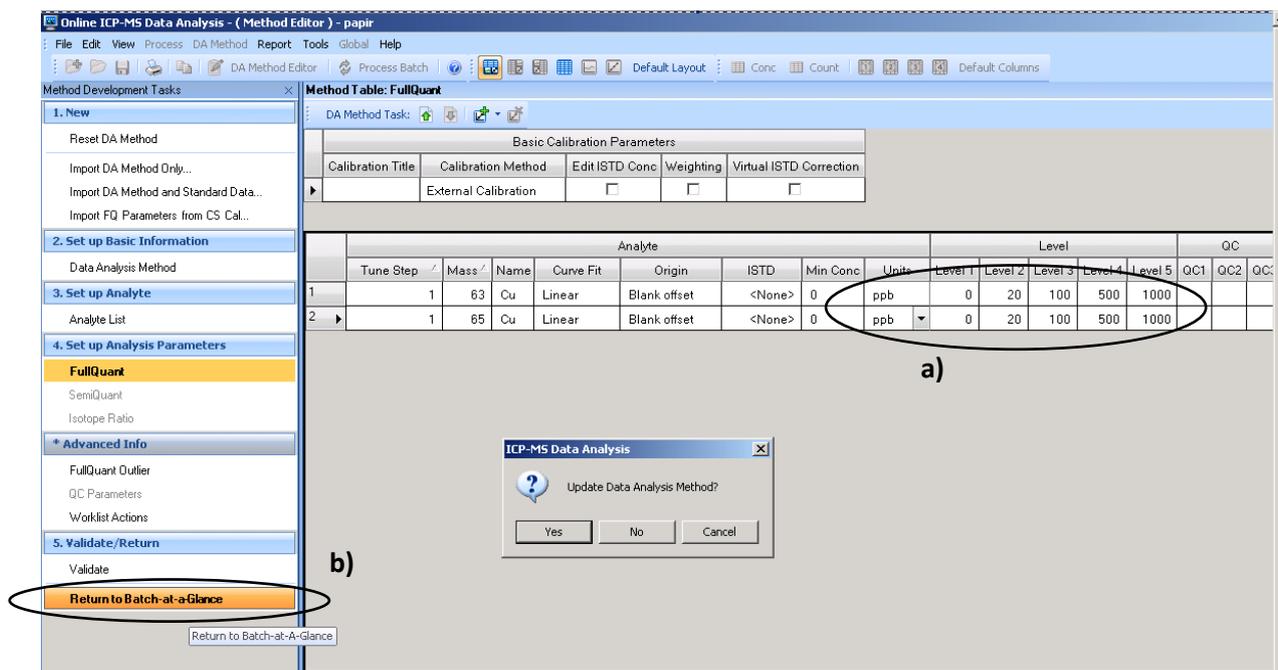
Fig. 9: Setting the calibration parameters.

➢ Preparation of calibration standards

We will use the methods of calibration curve for quantification of copper in ink (with respect to Zinc as internal standard). It is necessary to utilize internal standard because of matrix effect which can lead to signal distortion. It is chosen according these criteria: a) similar atom mass b) it is not present in the sample c) degree of ionisation in ICP is similar.

Prepare copper calibration standards with concentration 0, 100, 500 a 1000 µg/l in 100 ml volumetric flask from stock solution with concentration Cu 1 g/l. Next prepare zinc internal standard with concentration 200 µg/l from stock (1 g/l). Fill the values in the table 1.

Cu				
Concentration [µg/l]	0	100	500	1000
Pipetted volume[µl]				
Zn (internal standard)				
Concentration [µg/l]	200			
Pipetted volume [µl]				

Table 1: Calculation of addition to calibration and internal standards.

➢ Samples

Sample which was prepared in cryogenic mill and after microwave mineralization is necessary to dilute 10x.

➢ Start run

Now start run in program ICP-MS-Top → *Method → Run → Browse (find your folder) → OK → name your measurement* (only 7 letters) → in Sample Type click *CalStd* (Fig. 10) → Cal Level → 1 (measure the lowest concentration calibration standard) → *Run Method* → put the tube in your sample → *OK*. Mearsure all calibration standards according this procedure and increase the Cal Level. Then measure blank (10 x) and your sample (3x). See instruction ICP-OES for statistical evaluation.
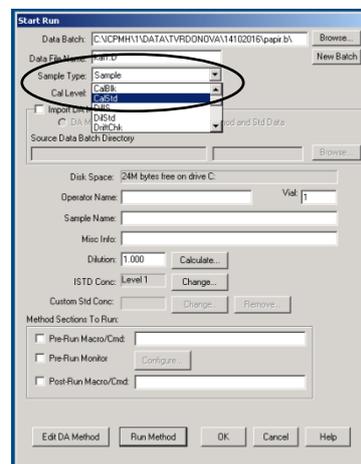
Fig. 10: Start Run.

➢ Data export

Export the table of your masured data (Fig 11) in program ICP-MS Data Analysis → *file → export → export table →* save in your own folder.
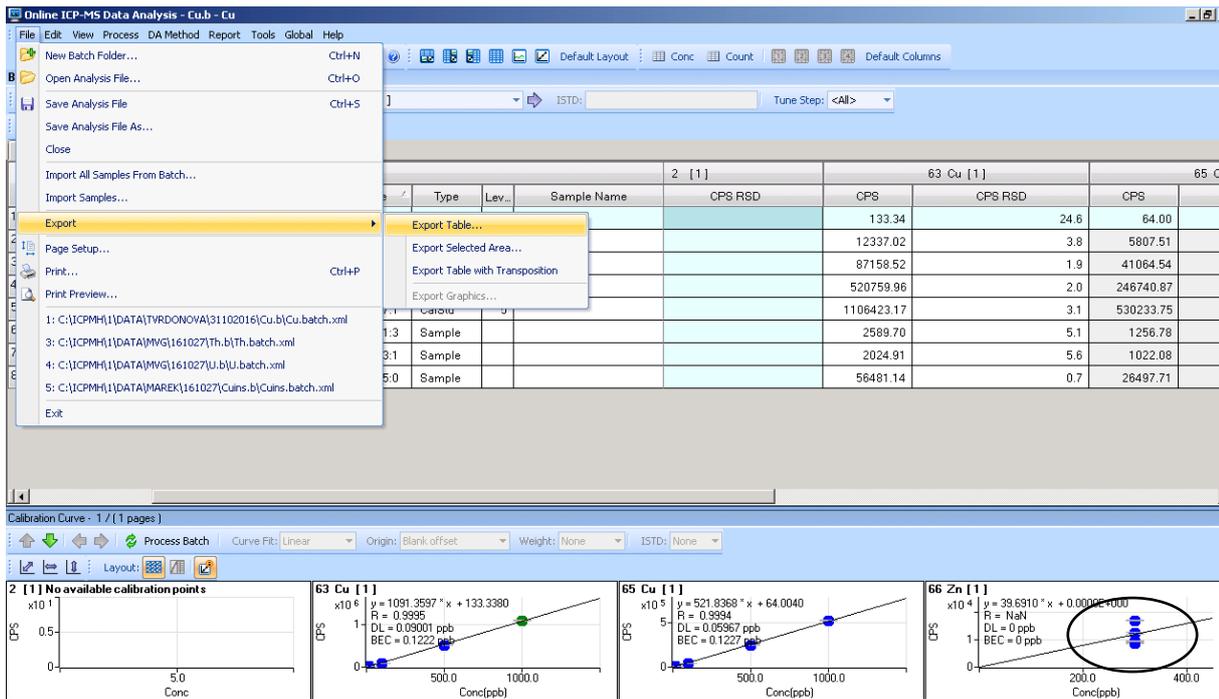
Fig. 11: data export.

➢ **Evaluation:**

Calculate the concentration of copper with respect to dilution, blank and internal standard (zinc) and convert it to the amount of copper (m_{tot}) in [μg] in ink on whole paper surface (100 cm^2) and then to the amount of copper in one shot of laser (m_{Cu}) [fg] (see instruction for LA-ICP-MS).

Correction of copper concentration with respect to internal standard.

$$c_r^{Cu} = \frac{c_r^{Zn}}{c_n^{Zn}} \cdot c_n^{Cu}$$

WHERE:

c_r^{Cu} real copper concentration Cu

c_r^{Zn} real zinc concentration – average of measured (Fig. 11).

c_n^{Cu} concentration of copper which you measured in sample

c_n^{Zn} concentration of zinc which you measured in sample

Total copper content [µg] in ink on whole paper surface (100 cm^2):

$$m_{tot} = \frac{c_r^{Cu} \cdot V}{m_{MW}} \cdot m_{cryo}$$

Where:

m_{tot} total copper content [µg]

V sample volume in volumetric flask

m_{MW} sample weight before microwave decomposition

m_{cryo} sample weight before cryogenic decomposition

Now convert it to amount of copper in one shot of laser m_{Cu} [fg] see instruction LA-ICP-MS, quotation 2 and then to [fg / cm^2]. Compare it with solution analysis by ICP-OES via statistic evaluation (see. Instruction ICP-OES). LOD convert to the paper surface.

Lab report has to contain:

- Calculation of copper concentration in sample.
- Total content of copper (include the calculations) in ink on whole paper surface in µg/cm^2.
- Statistical comparison of two solution analysis methods (ICP-MS a ICP-OES) via LOD.
- Evaluate your results.

Laser Ablation based methods: LA-ICP-MS

Imaging of elements in biological tissues by means of LA-ICP-MS

Imaging of elements in biological tissues is one of the most frequent application laser ablation technique combined with mass spectrometry of inductively coupled plasma (LA-ICP-MS). In the last decades, this analytical technique was developed as a powerful elemental bioimaging method with very good limits of detection (sub µg/g) and high spatial resolution (micrometer scale). It can open the way to an understanding of the transport of essential or trace elements such as Cu, Zn or Pt from cytostatic drugs during the treatment period.

The aim of this practicum is:

f) to optimize the lateral distribution (a choice of suitable scan speed)
g) preparation of set of calibration standards based on an agarose gel
h) to image and quantify the distribution of Cu, Zn and Pt in tumour tissue from a mouse treated by platinum cytostatic drug
i) to calculate the limit of detection (LOD)
j) to compare the LOD with another imaging method – LIBS.

1) Principle

The LA-ICP-MS setup consists of laser ablation system used for creation dry aerosol removed from the sample and ICP-MS used for the elemental analysis of the ablated material. . Focusing laser beam interacts with the sample surface, then the ablated material is transported by a carrier gas (He) as a dry aerosol to the ICP-MS. The plasma is a high energetic source and the ablated material is atomized, excited and ionized. Resulting ions enter into the mass spectrometer where are separated according to their mass to charge ratio (m/z) and detected by the electron multiplier.

Laser ablation
There are several processes when the laser beam interacts with the sample surface. The sample surface is sharply warmed even in particular depth under the sample. A sudden increase in pressure cases an explosion and a destruction of the surface layer (Fig. 1a) and evaporated and atomized material absorbs further laser radiation with subsequent creation of excited atoms and ions from the sample and surrounding gas which results in a creation of laser induced plasma and ablated material. The particle size distribution of the ablated **material** is dependent on laser ablation parameters such as are wavelength of the laser radiation, its laser beam fluence and repetition rate. In most cases the particle size distribution has bimodal distribution (Fig. 1b) when the largest amount of particles is in range of tens nm. This particles are created at laser ablation process and was not agglomerate into larger particle. The second maximum of the distribution is about hundreds nm and this particles

were created by agglomeration of smaller particles. The large particles are not atomized completely in comparison with the smaller one and it results in changes of ICP-MS signal.
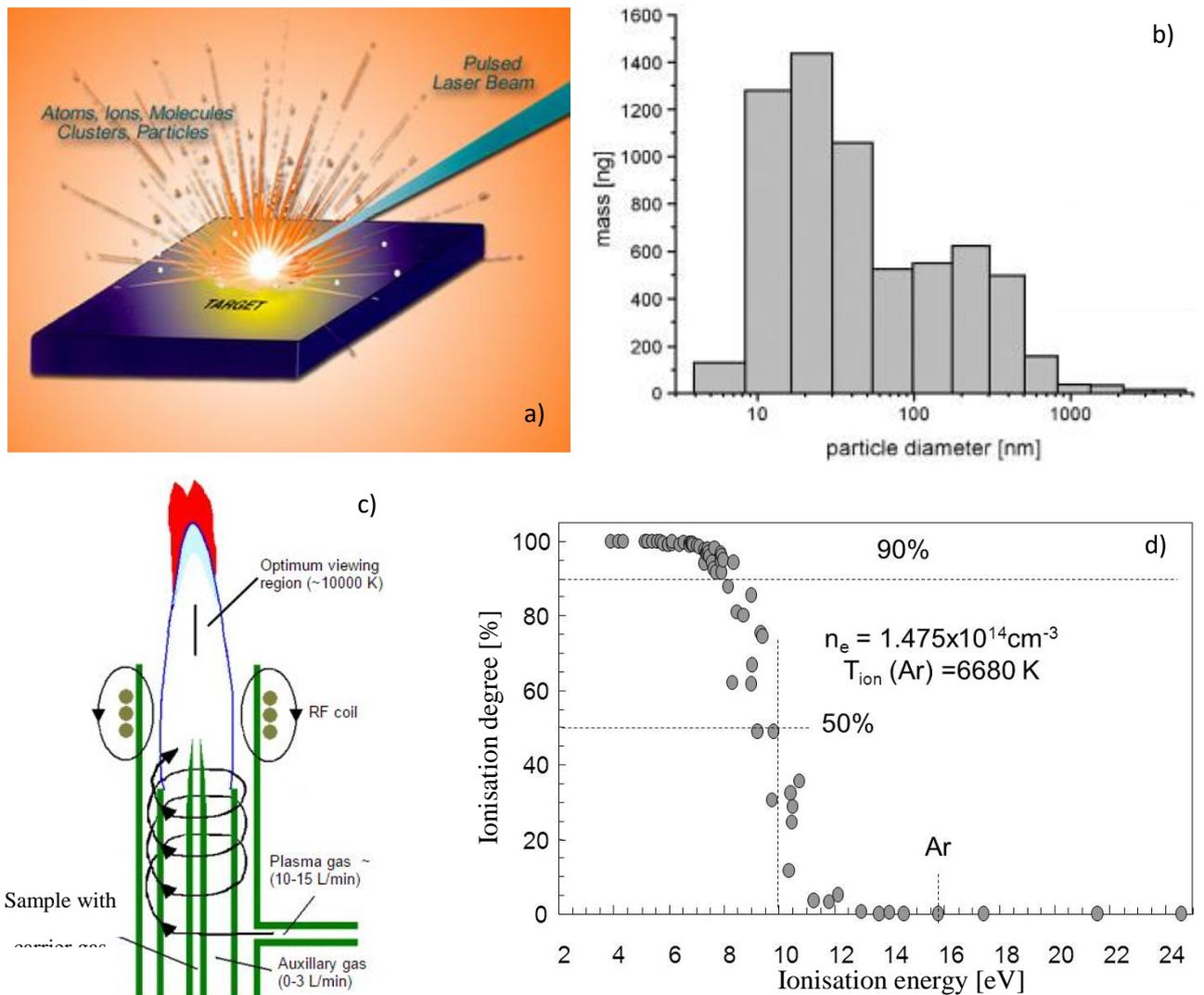

Fig. 1: a) Laser interaction with sample surface
http://blogs.maryville.edu/aas/files/2013/06/ablation_diagram.jpg
    b) Particle size distribution formed by laser ablation
(https://media.springernature.com/lw685/springer-static/image/art%3A10.1038%2Fs41598-017-03275-x/MediaObjects/41598_2017_3275_Fig4_HTML.jpg)
    c) Inductively coupled plasma
(https://chem.libretexts.org/@api/deki/files/100468/icp-use.png?revision=1&size=bestfit&width=309&height=451)
    d) Dependence of ionisation degree to ionisation energy
 (http://slideplayer.cz/slide/4108925/12/images/9/Ioniza%C4%8Dn%C3%AD+energie+(eV).jpg)

Inductively coupled plasma

Aerosol of the sample created by laser ablation is transported by carrier gas into the plasma torch, where (in high frequency magnetic field) argon plasma is created. Argon plasma is formed by plasma and auxiliary gas (Fig. 2c). Plasma is said to be the fourth state of matter due to its properties, which are very different from liquid or gas.

Definition:

Plasma is quasineutral gas of charged and uncharged particles, exhibits collection behaviour. Collective behaviour is a movement of particles depending on local conditions and the state of plasma in remote area.

Even though plasma can be created from any gas, noble gases are preferred thanks to their simple spectra, to not creating stable compounds and not dissociating the atoms. They have high of ionisation energy (Ar – first ionisation energy 15.8 eV) as well which leads to capability of ionisation of each element except for He, Ne and F (see Fig. 1d). Plasma temperature ranges between 6 000 – 10 000 K, depending on plasma region. Electron concentration in ICP is in range of $10^{20} - 10^{21}$ m^{-3} which is significantly higher amount than in flame ($10^{14} - 10^{17}$ m^{-3}).

Mass spectrometer

Ions created in the ICP source enter into mass spectrometer via interface system. The interface consists of sampler and skimmer cones and there is an effective separation of argon flow from atoms and ions flow occurred here. First the plasma beam (ions and atoms from the sample and carrier gas) go through the sampler cone into pre-vacuum space with pressure of 100 Pa, approximately. The supersonic expansion occurs with subsequent cooling of the plasma beam. Then it enters into ion optic via skimmer. The ion optic serves to focusing of the beam and removing of photons and non-charged atoms that would increase the background level of the detector. Focused ion beam enters into collision-reaction cell filled with He that suppress possible polyatomic interferences. Then the ions are separated in the quadrupole analyser according to their mass to charge ratio (m/z) and ions with selected m/z exit from the analyser and hit the electron multiplier that generates signal.

Fig. 2: Instrumentation of laser ablation with inductively coupled plasma and mass spectrometry (LA-ICP-MS). (D. Günther, B. Hattendorf, TrAC (2005), 24(3), 255-265).

2) Experimental Procedure:

- **Preparation of calibration standards**

Prepare 5 calibration agarose gel standards with addition of standard solution according the table 1.

	Agarose gel				
Addition of standard	**a**	**b**	**c**	**d**	**e**
Pt [$\mu g \cdot g^{-1}$]	0	1	2	10	20
Cu, Zn [$\mu g \cdot g^{-1}$]	0	10	20	50	100

Tab. 1: addition of standard to agarose gels.

Prepare the working solution (100 ml) that contains 100 mg $\cdot$ l^{-1} of Pt, Cu, Zn from a stock solutions 1 with concentration of 1 g $\cdot$ l^{-1}. Calculated volume [μl] of the addition from working solution 2 add to 0.1 g agarose and add up to 5 ml of MQ water in small beaker. Then put it on the magnetic hotplate stirrer and boil it weakly under constant stirring until it becomes homogeneous (5 min, approx., see

Fig. 3). 100 μl of mixture pipette on the small glass slide and let to gel. Then attach the 5 small glasses to the large glass slide with the double sided adhesive tape.
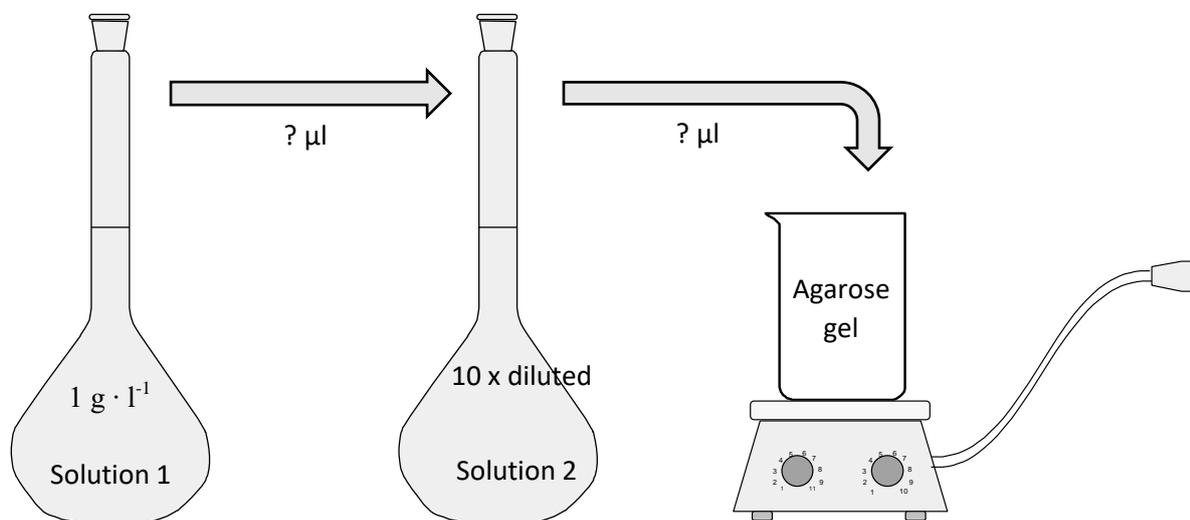
Fig. 3: Preparation of calibration standards.

	Agarose gel				
Standard addition	**a**	**b**	**c**	**d**	**e**
Pt [μl]	-				
Cu, Zn [μl]	-				
Water [ml]	5				

Tab. 2: Calculated standard addition [μl] to agarose gels.

Fill the calculated amount of working solutions into Tab.2.

- **Sample handling**

Remove the sample holder from the ablation chamber and put the sample of the tissue and glass with calibration standard on it (Fig. 4) and attach it with the double sided adhesive tape to secure against a movement of the sample in ablation chamber during laser ablation.
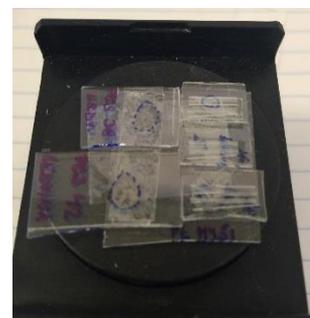
Fig. 4: Sample handling.

3) LA-ICP-MS Measurement

Turning on the machines:

Turn the argon and helium (Fig. 5a) valves on the wall so that the machines have access to a flow of the gases. Turn on the cooling system (Fig. 5b). Now open the valve on the helium cylinder (Fig. 5c). In order to turn on the laser, flip the switch on the back of the machine (Fig. 5d) and turn the key found under the machine (Fig. 5e). Turn on the flow meter. Finally, turn on the computer.

Now open the desktop programs New Wave Research – Laser Ablation, ICP-MS-Top, and Smart Control. On the Smart Control system select file, open, and then find the serial number of the flowmeter. Now click OK. On this display 100% flow is a flow rate of 2.00 L/min.
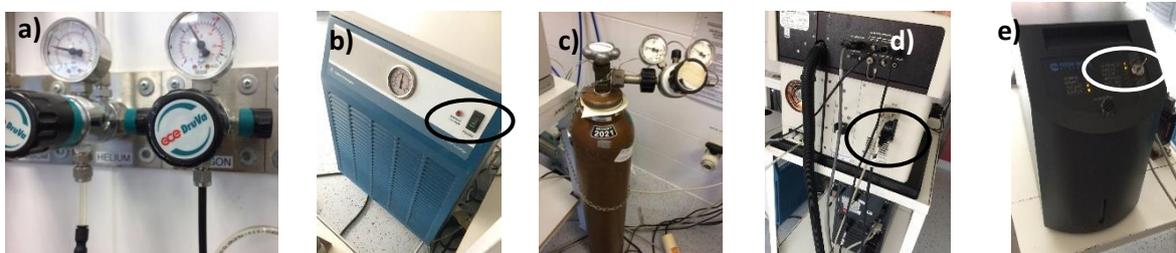
Fig. 5: Turn on of individual parts of instrument LA-ICP-MS, a) argon, helium, b) cooling, c) helium to collision-reaction cell, d) and e) laser.

The air in the ablation chamber must be flushed after the ablation chamber is opened. In order to do this, go to the New Wave Research program and switch the system from *bypass mode* to *purge mode* as shown in figure 6.
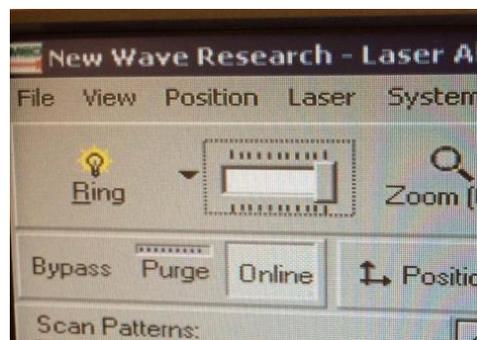
Fig. 6: Switching the mode from bypass to purge to online.

Now move to the Smart Control program and increase the Helium flow to 50% (1.00 L/min). Allow the ablation chamber to be washed for approximately 1 minute and just before the ablation chamber moves from the *purge mode* to the *online mode*, reduce the He flow to 1%.

ICP-MS-Top

In order to start ICP move to the ICP-MS-Top program and click on the *plasma on* icon. The plasma igniting should be visible on the Mass Spectrometer (Fig. 7). Wait for 45 minutes after the plasma is ignited to start measurements. During the waiting period, it is a good idea to start setting up the methods.

Fig. 7: Ignited plasma in the Mass Spectrometer.

In the folder data and methods create new folder and name the folder after the analysis date. Now the ICP-MS-Top measurement conditions must be set.

Methods → Edit entire methods → select Method information a Aquisition → ok → create a description of the analysis being performed *→ Time Resolved Analysis → ok → periodic table* (select the elements being analyzed) *→* the *integration time* for each element should be as follows: Carbon 0.01s, Silicon 0.1s, Copper 0.1s, Zinc 0.1s, Platinum 0.3s. *→ Real Time Plot* select *extract → Time window → 500 s → OK → Aquisition → 9990 s (max. time analysis) → OK.*

ICP-MS Data Analysis

Once the data analysis program opens select the folder icon with a plus mark. Click data. Name the folder. Click create. Now go to the *DA Method* tab. *→* c*lick edit → Analys. Mode. → select Timechard* (Fig. 8). Then click the *green arrow icon. →* Load List with element of interest *→* Methods and select the name of the folder (Fig. 8). Click *Batch-at-a-glance* on the right *→ yes.*
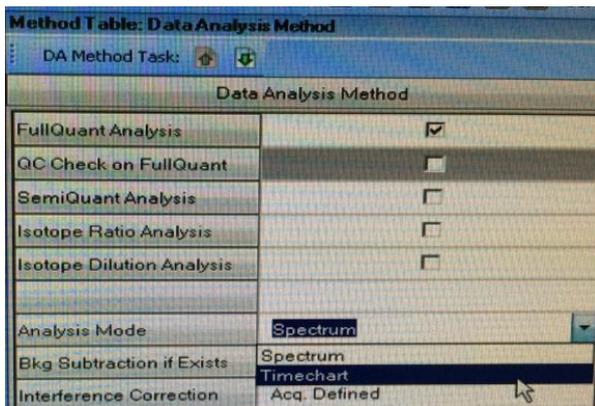
Fig. 8: Editing the data analysis information.   *Fig. 9: Selecting the correct folder for data analysis.*
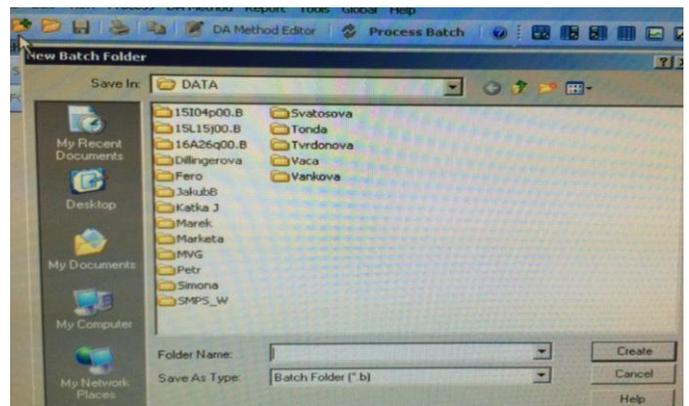
New Wave Research

Now move to the New Wave Research software. Set the frequency to 10 Hz (fig. 10a), the laser beam size to 100 μm (fig. 10b), the energy to 96 % (cca 8 J/cm^2) (fig. 10c).
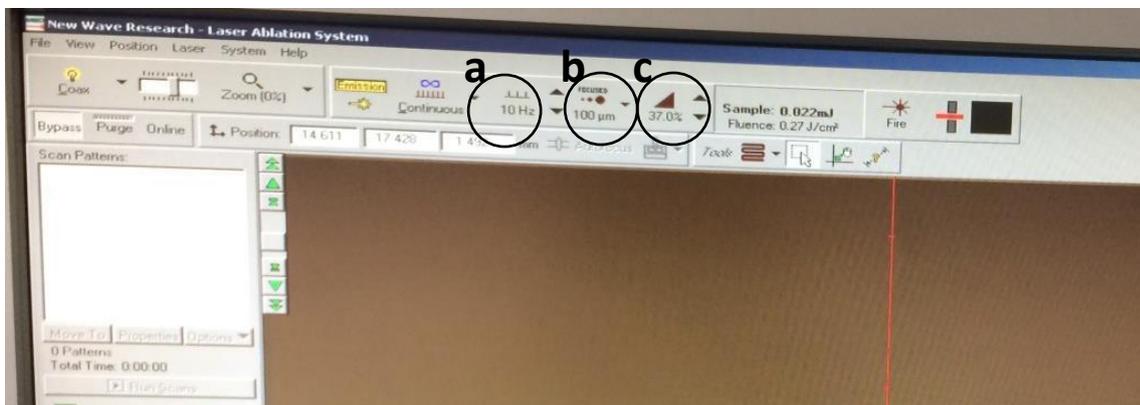
Fig. 10: a) Frequency, b) laser beam size, c) energy percentage

Now it is necessary to prepare an ablation pattern for each sample. Using the microscope find the outline of the tissue sample and note the leftmost, rightmost, topmost, and bottommost points of the tissue. Now click tools, select line and create a line on the page (left click, drag, left click, right click). On the left-hand side of the screen under *scan patterns* select the line just created and right click (Fig. 11a). Now click on *edit endpoints* (Fig. 11b). Edit the X coordinates to be the leftmost and rightmost points. Edit both the Y coordinates to be the topmost point. Click *OK*. Now right click the line again and select *properties*. Change the scan speed to 200 μm/sec (Fig. 12a), the *output energy* to 96 % (Fig. 12b), the *spot size* to 100 μm (Fig. 12c), and then check Default (Fig. 112d). Click *OK* (Fig. 12e).
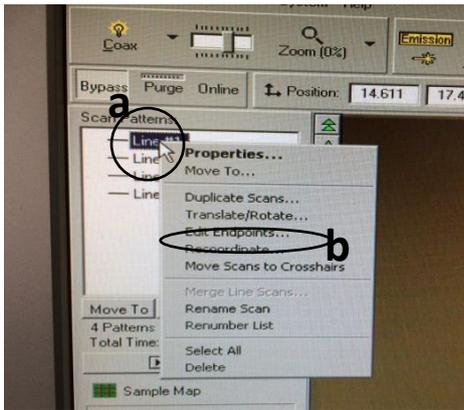
Fig. 11: a) Selecting the line, b) edit size, endpoints
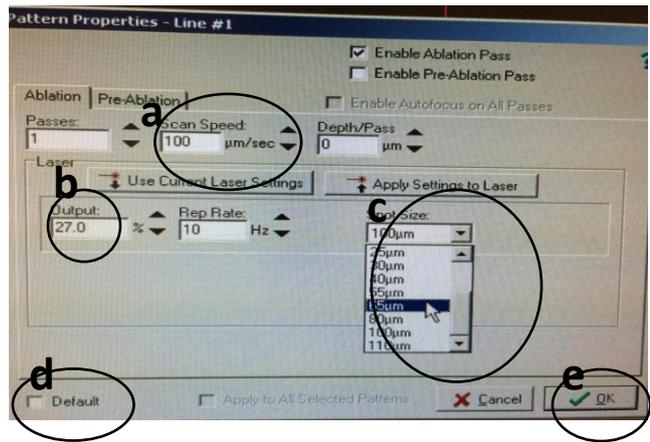
Fig. 12: a) scan speed, b) output energy, c) spot d) select default, e) OK

Right click the line again and select *Duplicate Scans*. Each line should be 0.11 mm apart, so based on the length of the tissue determine the number of copies necessary to create the grid (Fig. 13). Enter this number and click *OK*. Make sure that the grid encompasses the entire tissue area.
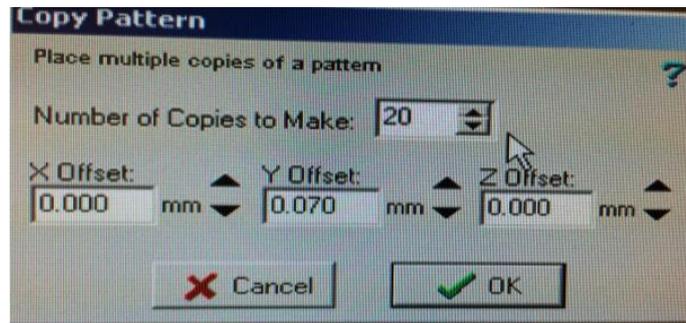
Fig. 13: Adjusting the copy pattern.

One line for each calibration standard must also be created. Each line should be at least 2 mm in width of the calibration gel.

Now go back to **ICP-MS-Top** software and select *Method* and then *Run*. Choose where the results should be saved. Click *OK*. Name the measurement (only use 7 letters). Click *Run Method*. The ICP-MS-Top software will start collecting data and should show data looking similar to figure 14.

In the **New Wave Research** program click *Run Experiment* and select *Enable Laser During Scans*. Change the laser warm up time to 5 seconds. Then click *Run*.
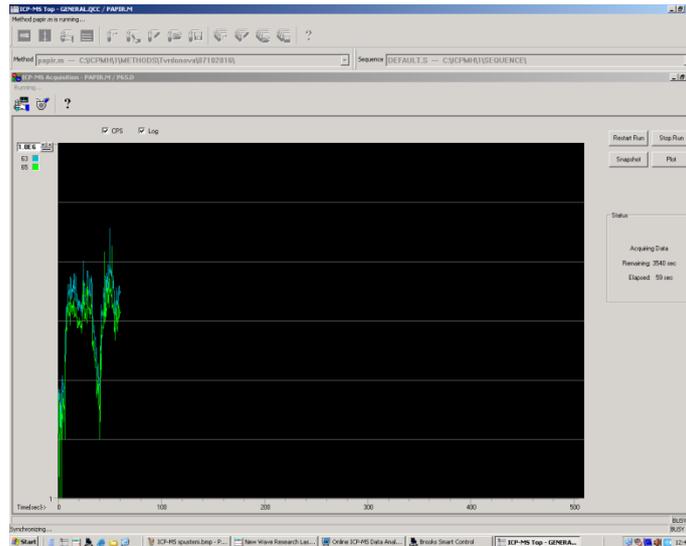
Fig. 14: ICP-MS-Top software after starting to run the method.

Once the laser has gone through the entire ablation pattern click *Stop Run* in the **ICP-MS Top program**.

Importing the Results:

Using the ICP-MS Data Analysis program and select *File → import samples*, select the folder made earlier, and click *OK*. On the chart below right click and select *Tabulate Chart* and then select *csv Data* (Fig. 15).
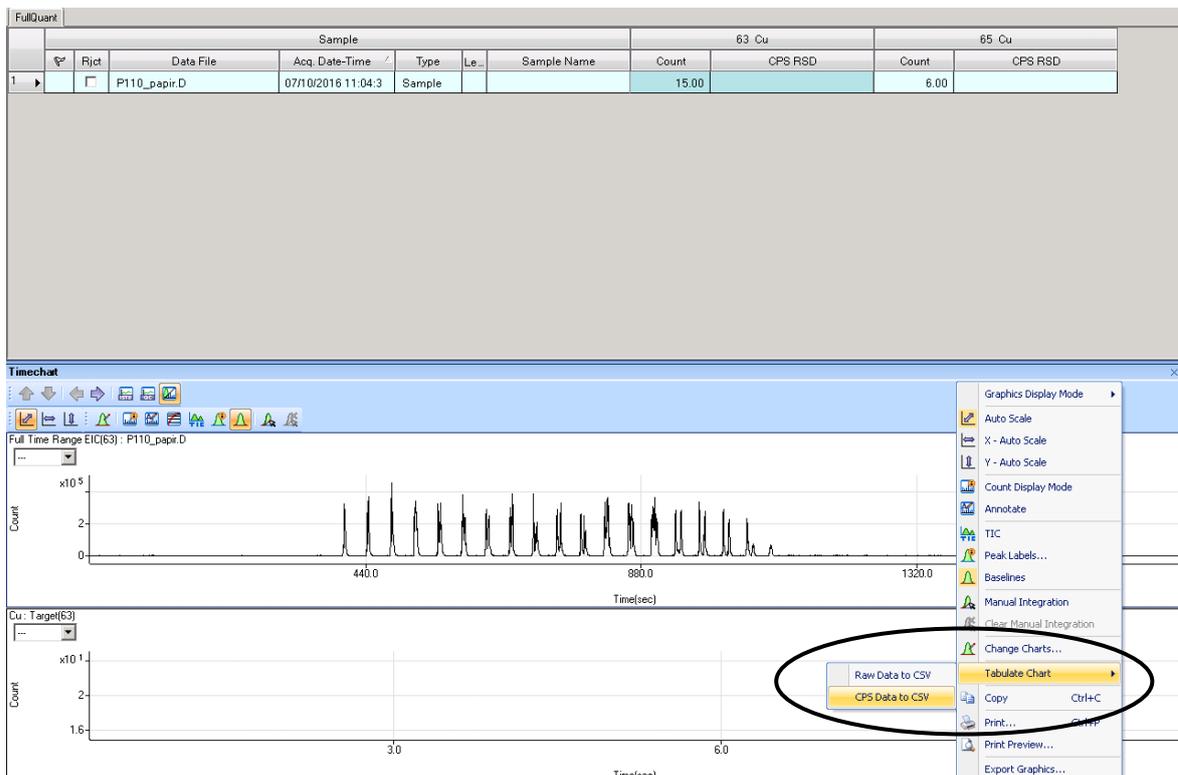
Fig. 15: Converting the data into csv file.

30

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The data collected should look similar to the data in figure 16.
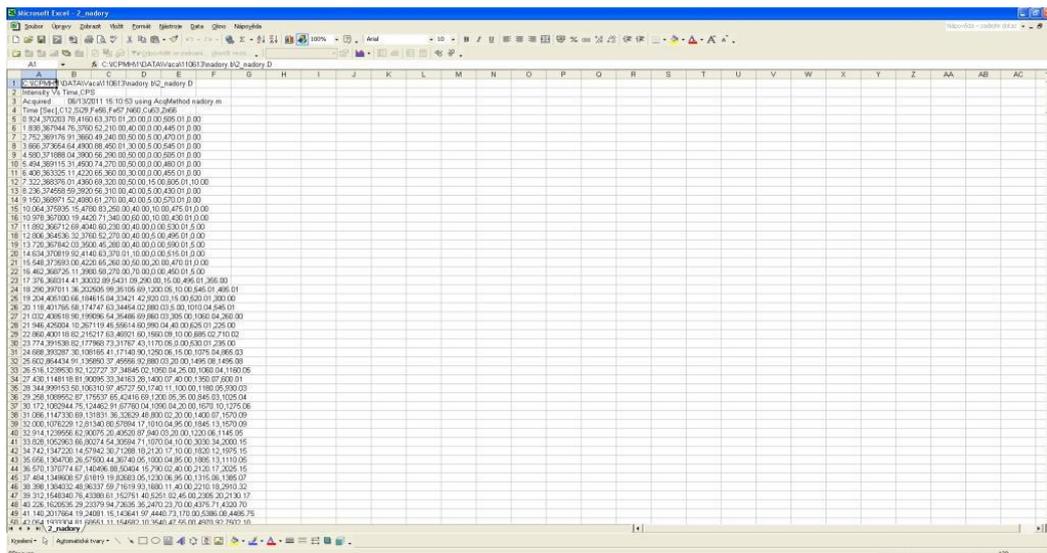
Fig. 16: Data in converted from the ICP-MS data analysis to csv format.

Turning off the device:

In the ICP-MS-Top program click the *plasma off* icon at the top left portion of the screen. Now close all programs and shut down the computer, the cooler, and the laser. The gases must also be switched off.

Creating the maps:

Start by opening the **Laser Ablation Tool program**. Click *load data* from selected file and choose the csv file. Click *next*. Now sync one of the elements by setting the start time of the measurement. Next set the rising and falling steps as well as the period of signal. Continue adjusting these settings until the intervals on the graph turn gray, as shown in figure 17.

Click *next* and set the *speed of the laser* and the *distance* between the two laser tracks (the distance should be 110 μm). Then click *finish*.

To save the collected data click the *XLS* icon at the top left corner, name the file, and save it.

Now open the file in **excel**. There should be an excel sheet for each element. Select an element and highlight all of the data. Then, go to insert and under graphs click on templates. Select the template with the icon shown in figure 18.
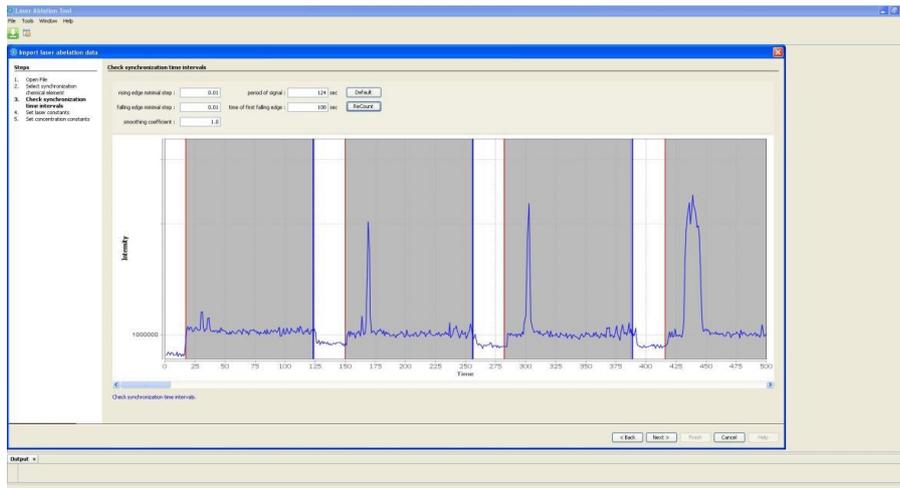
Fig. 17: Using the laser ablation tool to sync each element.
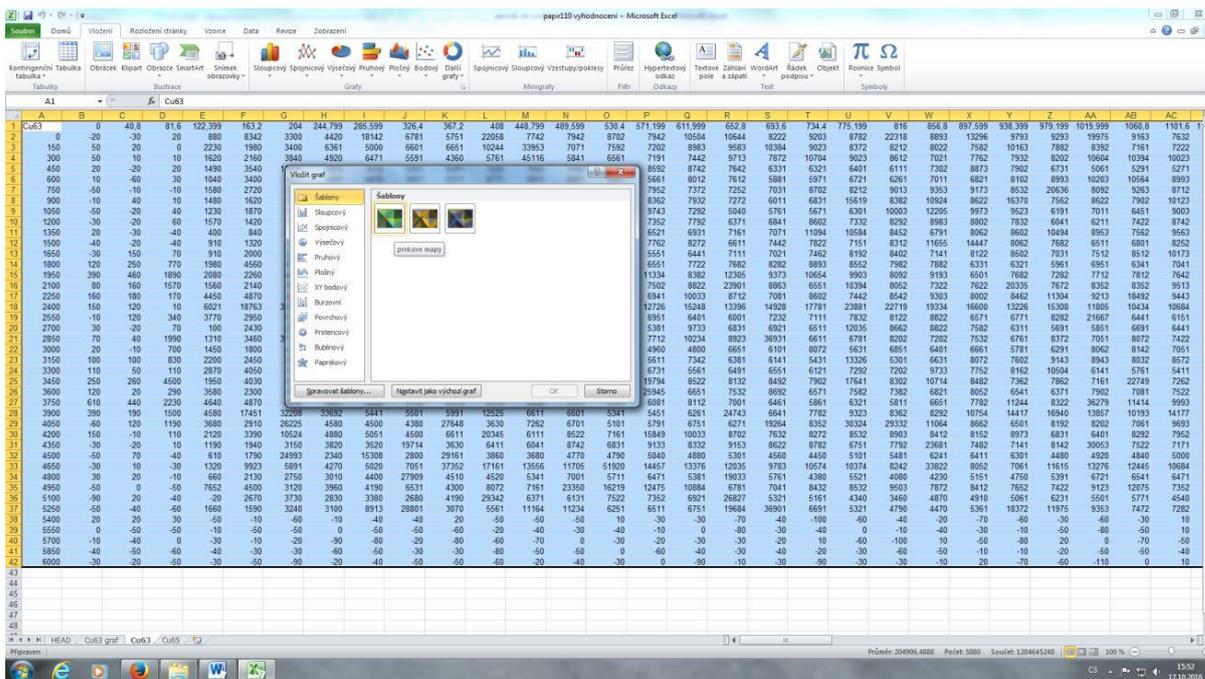
Fig. 18: Selecting an appropriate template for the map.

Once the graph is created, right click, click *move graph → new sheet →* and then name the graph. Next, right click on the edge of the graph and click on *format axis*. Adjust the minimum and maximum values to create an appropriate range for the legend. The main unit should be one order lower than the maximum.

Now, use the *cutting tool* on the computer to cut out the graph from the excel sheet. Save the image and then open it with the program **XnView** (any other enabling basic image processing). It is necessary to adjust the aspect ratios of image (see excel μm x μm) → *picture → size →* (Fig. 19) and save it. Create panoramic picture with scale → *panorama → add* (Fig. 20a) → select our picture → *add* (Fig. 20b) → *add* scale picture as well → *ok* (Fig. 20c) → *create* (Fig. 20d).
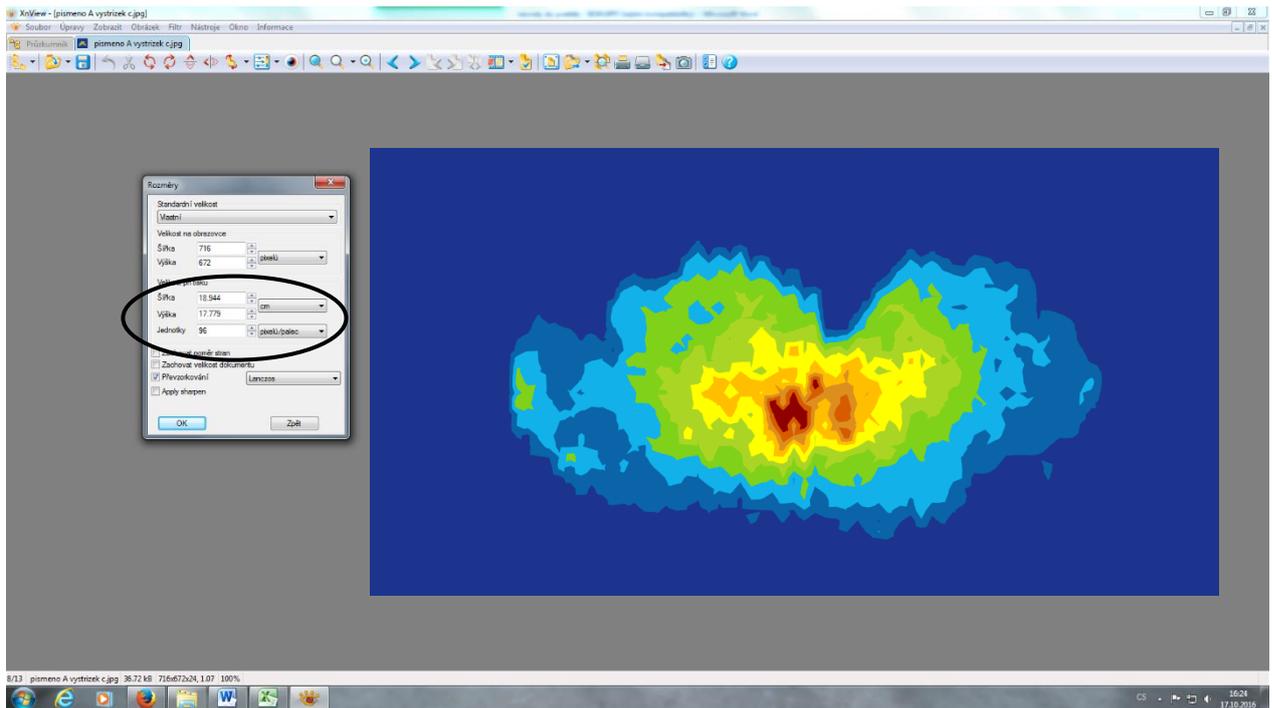
Fig. 19: Correction of picture aspect ratio.
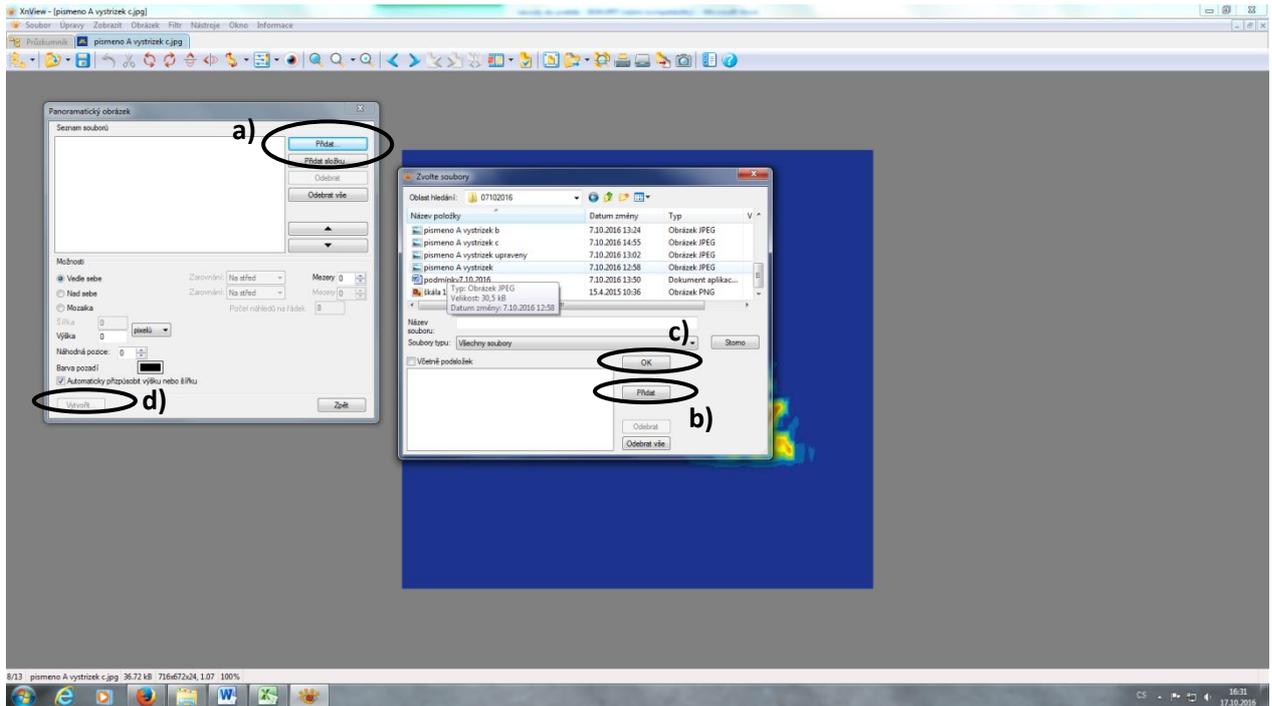
Fig. 20: Creation of panoramic picture.

At the end insert the describtion of scale (units, name of sample, etc.) → picture → *add text* → *write a text* (Fig. 21) → *ok* → use the mouse to select the position of text in picture → save picture.
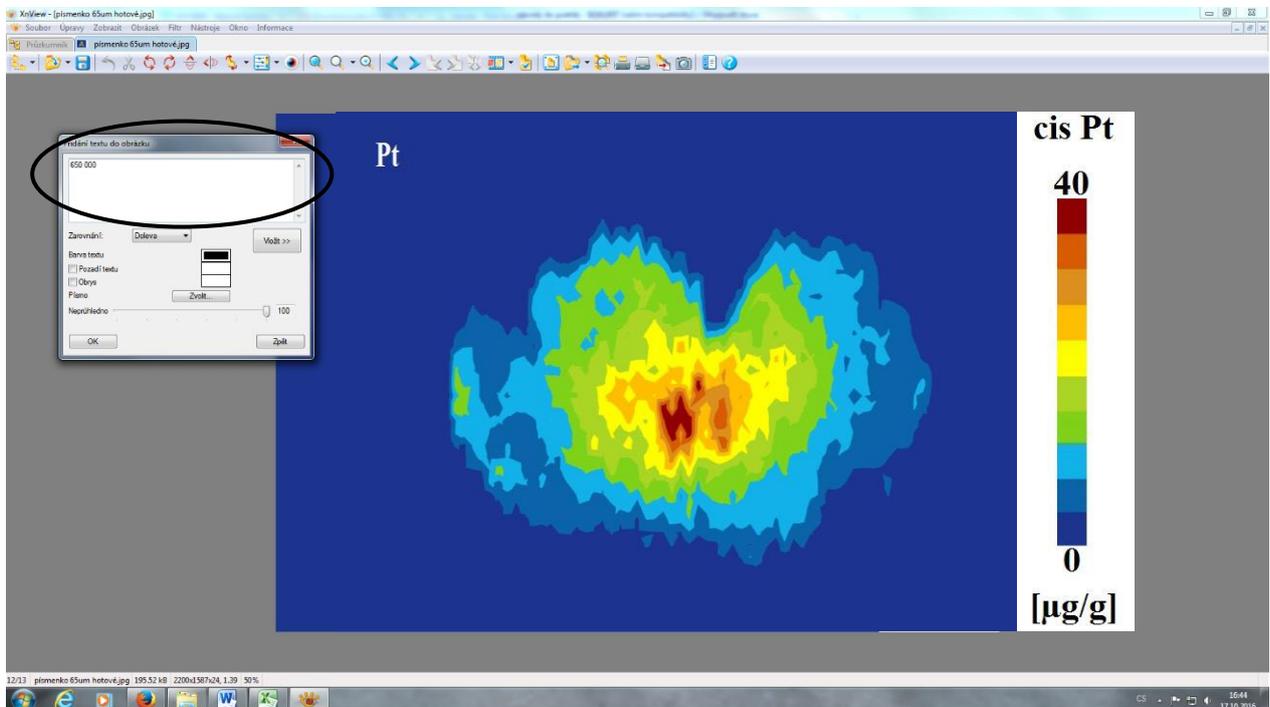
Fig. 21: Description of scale.

EVALUATION:

➢ There are 2 very important parameters which have to be optimize before you start to measure the real samples. **Scan speed** [μm · s⁻¹] influence the **a) relative broadening Δw_rel** (equation 5) end thereby lateral resolution and **b) time of analysis**. One of the other important parameter is **c) sensitivity** which is expressed by **limit of detection (LOD).** You will calculate LOD_1 of Cu in ink to compare LIBS and LA-ICP-MS and $LOD_{2,3,4}$ of tissue sample for each determined element (Cu, Pt, Zn).

- **Amount of copper [fg] in one shot of laser:**

It is known the total copper content (m_{tot}) on the paper (S_{tot} - Fig. 23) determined by solution analysis via ICP-MS. Now convert it to the copper amount in one shot of laser (m_{Cu}) according the equation (1) and (2). Diameter of laser spot is 100 μm.
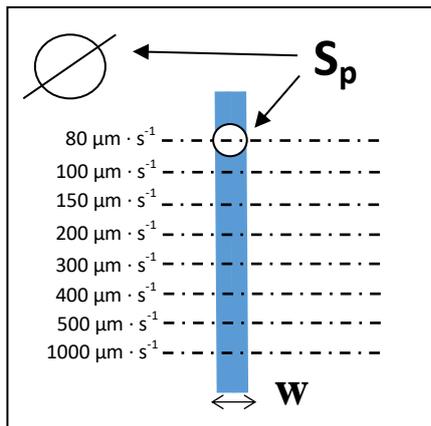
Fig. 22: Different scan speed line by line.

$$S_p = \pi r^2 = 3,14 \cdot 50^2 = 7854 \, \mu m^2$$
$$(1)$$

$$m_{Cu} = \frac{S_p \cdot m_{tot}}{S_{tot}}$$
$$(2)$$
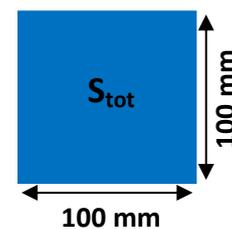
Fig. 23: Original paper size.

- **Limit of detection (ink):**

Calculate for each scan speed and compare (fig. 22).

$$LOD_1 = \frac{3 \cdot SD \cdot m_{Cu}}{I_{Cu}} \tag{3}$$

WHERE:

SD   is standard deviation of background (10 values of background – intensity of copper before the starting ablation).

I_{Cu} is average intensity of Cu (5 values of intensity in ink line during the ablation)

m_{Cu} is amount of copper [fg] in one shot of laser (diameter of laser spot 100 µm).

Convert LOD_1 to [fg/µm^2].

- **Limit of detection in tissue sample:**

$$LOD_{Zn} = \frac{3 \cdot SD}{GL_{Zn}} \qquad (4)$$

WHERE:

SD   is standard deviation of background (10 values of background – intensity of zinc before the starting ablation).

GL_{Zn} is guideline of calibration curve (the average amount of the line intensities is plotted to *y*-axis and concentration of added amount [µg/g] of element of interest to *x*-axis).

- **Relative broadening (ΔW_{rel}):**

Lateral resolution of imaging is quantified as relative broadening (Δw_{rel}) of width ink line (*w*) – (fig. 22) measure it by microscope in program *New Wave Research - Laser Ablation System,*

$$\Delta W_{rel} = \frac{\Delta w_{app} - w}{w} \cdot 100 \qquad (5)$$

where w_{app} is measured width line and w is real width of the ink line (Fig. 24).
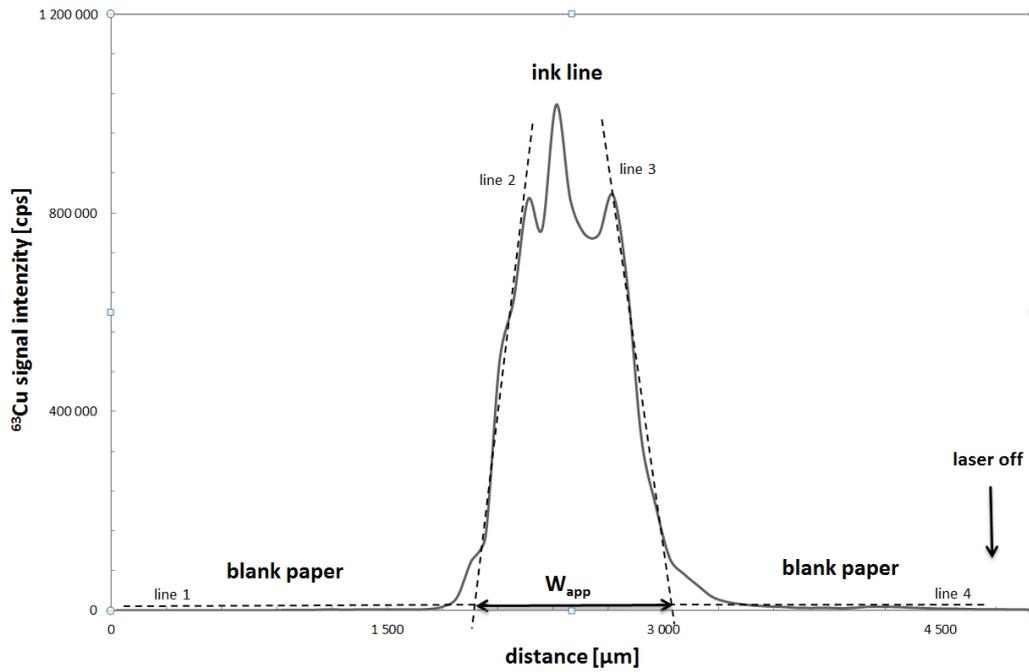
Fig. 24: Record of line scan speed 200 µm/s and the laser spot diameter 100 µm together with determination of apparent width W_{app} which was obtained as the trend intersections of line 1 and 2 and trend lines 3 and 4, respectively. These intersections indicate the onset and end points of ^{63}Cu signal intensity in ink line.

Lab report has to contain:

- Pictures of quantitative determination of copper and zinc distribution in tumour tissue.
- Calculation of LOD_1 [fg/µm^2], $LOD_{Zn,Cu,Pt}$ [µg/g] and relative broadening ΔW_{rel} [%]
- According LOD_1 and ΔW_{rel} compare two imaging method LA-ICP-MS and LIBS and with LOD_1 compare method of solution analysis (ICP-MS and ICP-OES) as well.

Laser-Induced Breakdown Spectroscopy LIBS

Determination of Cu in a blue print by LIBS technique

Principle
Laser-Induced Breakdown Spectroscopy (LIBS) is a method of atomic emission spectroscopy which uses radiation of microplasma created by the interaction of focused laser beam with the sample surface.

Laser beam reaching the sample surface causes rapid heating of the material and subsequent ablation. Small amount of the material is being released (in the order of tens to hundreds of ng) and a radiation of microplasma created is detected and evaluated. After the end of the pulse microplasma is expanding and continuous bremsstrahlung is emitted. After a moment in the order of hundreds of ns microplasma starts to cool down rapidly, the intensity of bremsstrahlung decreases and characteristic emission lines of the atoms and ions are left in the spectrum. It is possible to determine the elemental composition of the sample based on matching the lines observed to particular elements (Fig. 1).
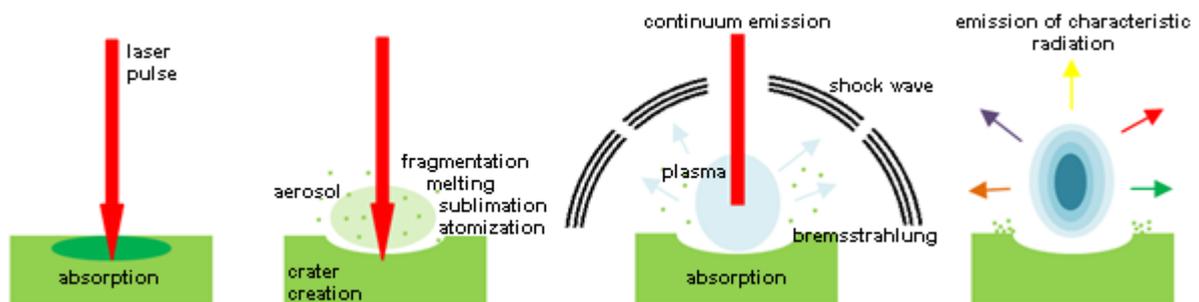
Fig. 1 Scheme of the processes in Laser-Induced Breakdown Spectroscopy [1]

The method doesn't require any sample preparation and it is possible to measure in the air at the atmospheric pressure. Possibility of remote measurements is among the advantages of this method, either with so called remote LIBS using an optical fibre or stand-off LIBS with a telescope. LIBS is microdestructive and, therefore, usable when other methods of sampling are too destructive.

Thanks to a good spatial resolution depth-resolved analysis (Fig. 2) can be carried out and maps of elemental distribution (Fig. 3) can be created. During the depth-resolved analysis the laser pulse is repeatedly directed to one point, one spectrum is acquired for each pulse and intensity of significant line as a function of number of pulses is observed. Depth profiling can be used e.g. to distinguish layers of multi-layered surfaces. Elemental distribution analysis is carried out in a pre-set grid of points in the area of interest. One pulse is directed to one point of the grid and a spectrum is acquired. Elements present in the area of interest can be given different colours and illustrative maps of their distribution can be created.
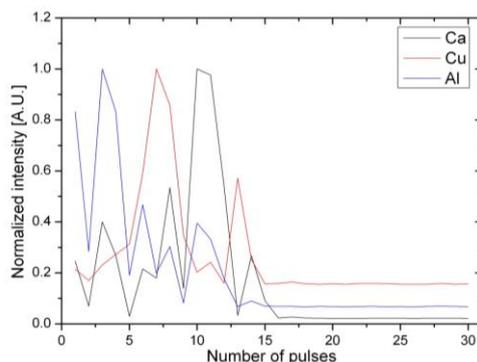
Fig. 2 Example of the depth profile of a three-layered sample *Fig. 3 Elemental distribution of Al*
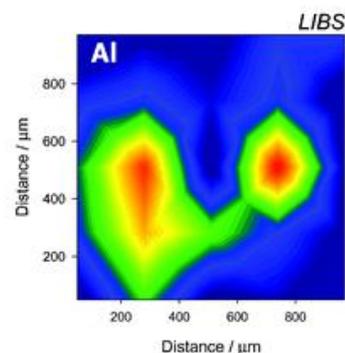
Instrumentation

Modified ablation system New Wawe UP-266 MACRO (Fig. 4) is equipped with a solid-state Nd:YAG laser operating at fourth harmonic frequency (~ 266 nm). The sample holder can be moved in *x* and *y* axes using a software, while the sample is recorded with a camera. That enables scanning of the sample surface. Measurements are usually carried out in the air at the atmospheric pressure. However, the environment of the sample and the pressure can be adjusted by using the ablation cell. Emission of microplasma is recorded and transmitted through an optical fibre to a Czerny-Turner monochromator (Jobin Yvon, TRIAX 320, Francie) and it is detected by an intensified CCD (iCCD) camera (PI-MAX 3, Princeton Instruments, USA). The intensity of the signal is then evaluated as a function of wavelength and can be further elaborated.

Fig. 4 New Wawe UP-266 MACRO

Turning-on:

1) Turn on the power supply of the laser system UP-266 MACRO
2) Turn on the laser UP-266 MACRO with a 0/I switch in the back
3) Turn on the Dell computer (black one)
4) Turn on the *MEO laser* program
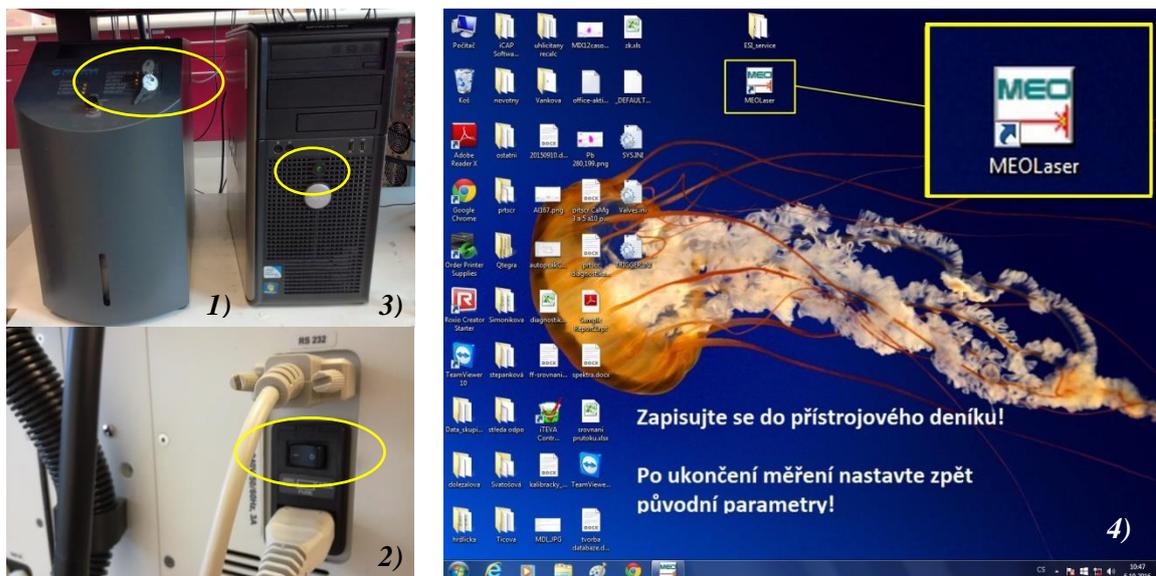
5) Turn on both delay generators by turning the POWER switch to ON

6) Turn on the pulse generator and divider, both values are pre-set to 005 (~ 2 Hz)
7) Turn on the source and the control unit of the detector using the 0/I switch in the back of the source
8) Turn on the HP computer (silver)
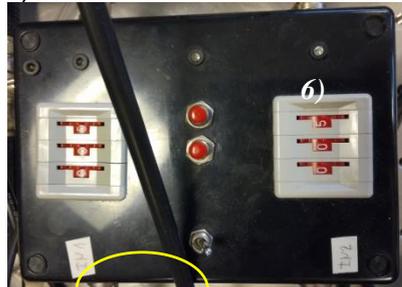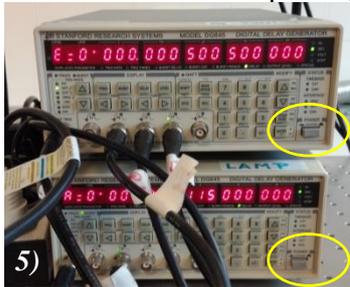
9) Turn on *Winspec* program, choose *Restore to last setting.*
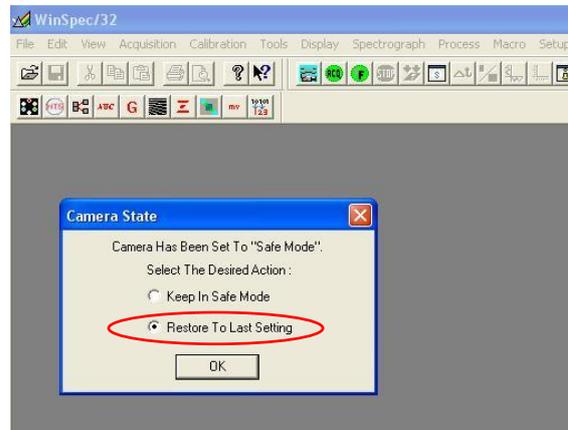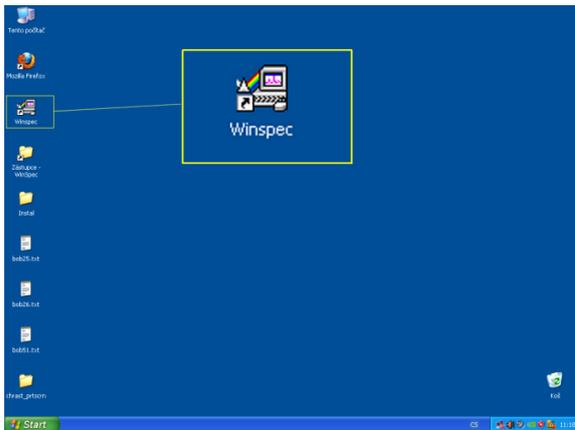
10) Turn on the ICCD camera using the ON/OFF switch in the back of the detector

Analysis procedure

1) Sample preparation
Cut out the printed sample. Adjust the sample to the base using a double-sided adhesive tape. Place the base onto the sample holder. Adjust the sample using the software so it is in the middle and parallel to the axes in the software.
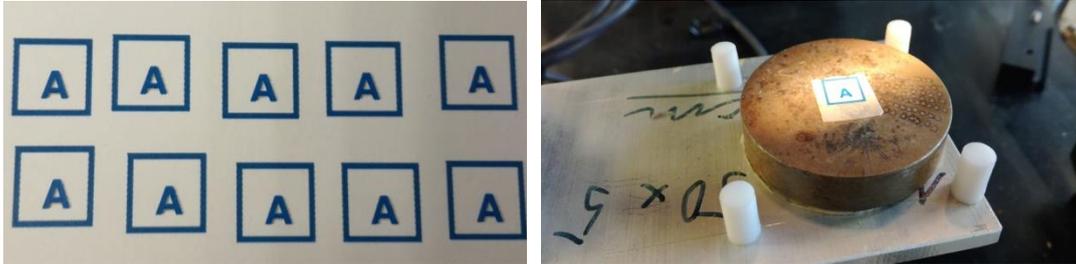
Turn on the light above the sample using the *Ring* mode
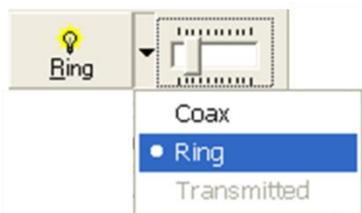
Focus the sample surface using the micro-shift screw.

Sample holder control:

Micro-shift screw for up and down movement

Arresting screw - first loosen it, set the sample, then tighten it again. Secure the sample holder with your hand the whole time!
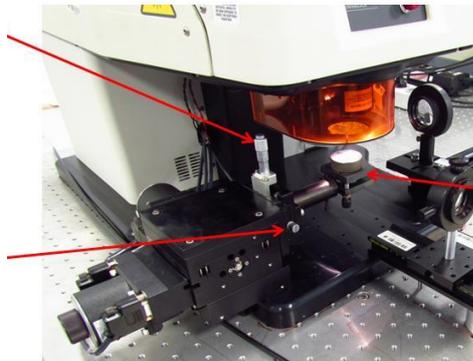
Sample holder with the screws to set the leaning angle of the sample
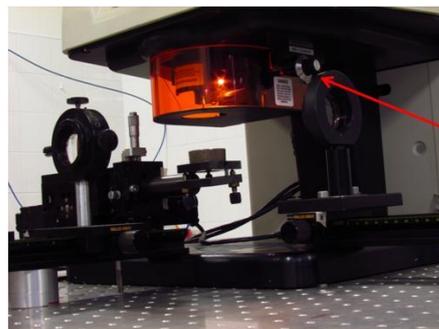
Revolving screw for focusing

2) Experimental set-up

Parameters of the spectrometer and the detector are set up first using the HP computer. Characteristic spectral lines of **copper** lie at **324.7 nm** and **327.4 nm**. Set the wavelength in the *Winspec* program to 327 nm. Choose *Spectrograph → Move…→ Move to: 327 nm.* Grating n. 2 (2400) and a 0,6 slit are already pre-set.
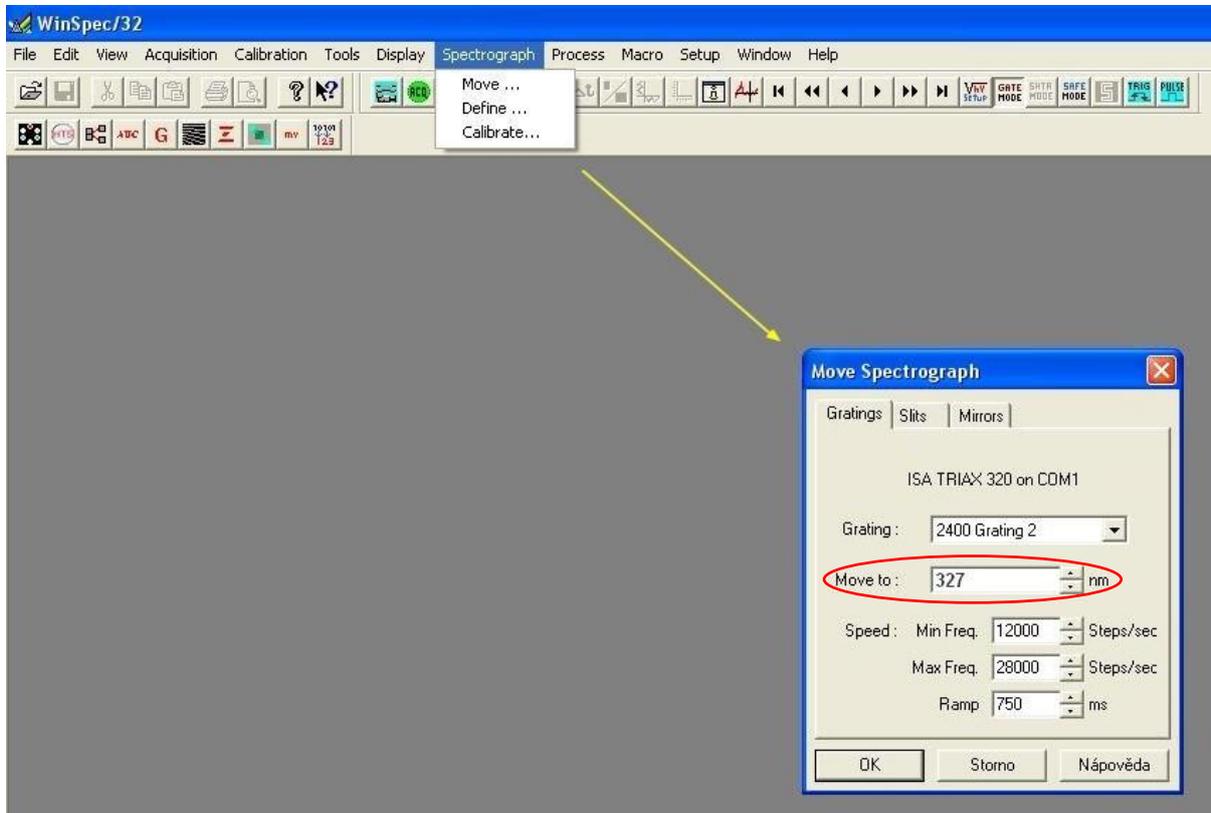
Choose the blue *Pulse* button and set the *Gate Width* to 5 μs and the *Gate Delay* to 0,5 μs. Press *Apply.*
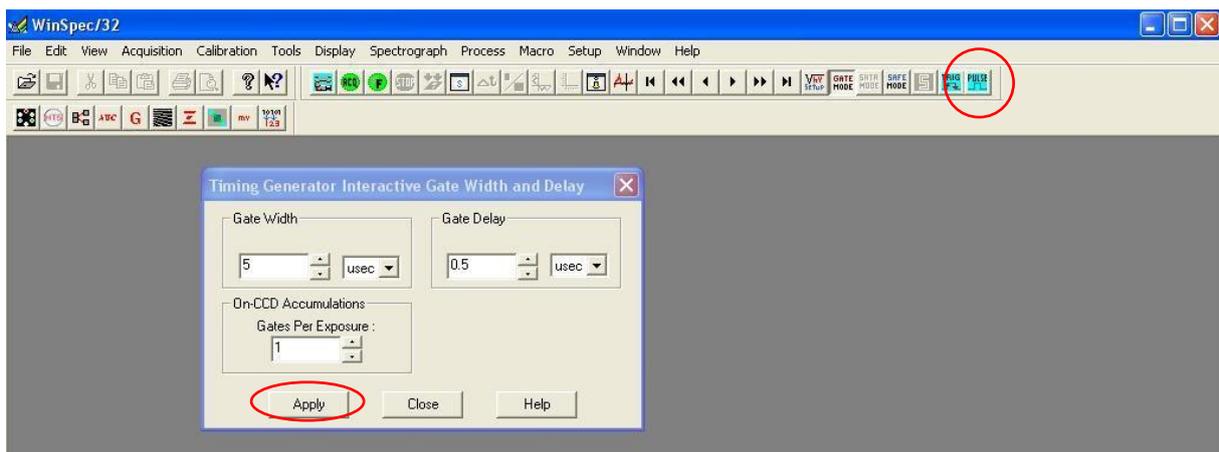
Choose *Acquisition → Experiment Setup… → tab Main → set Number of Spectra* to 1800.

… → tab *Data File* → set the *Name*: SESKUPIT in the file *C:\Program Files\Princeton Instruments\Winspec\Cviceni SESKUPIT*
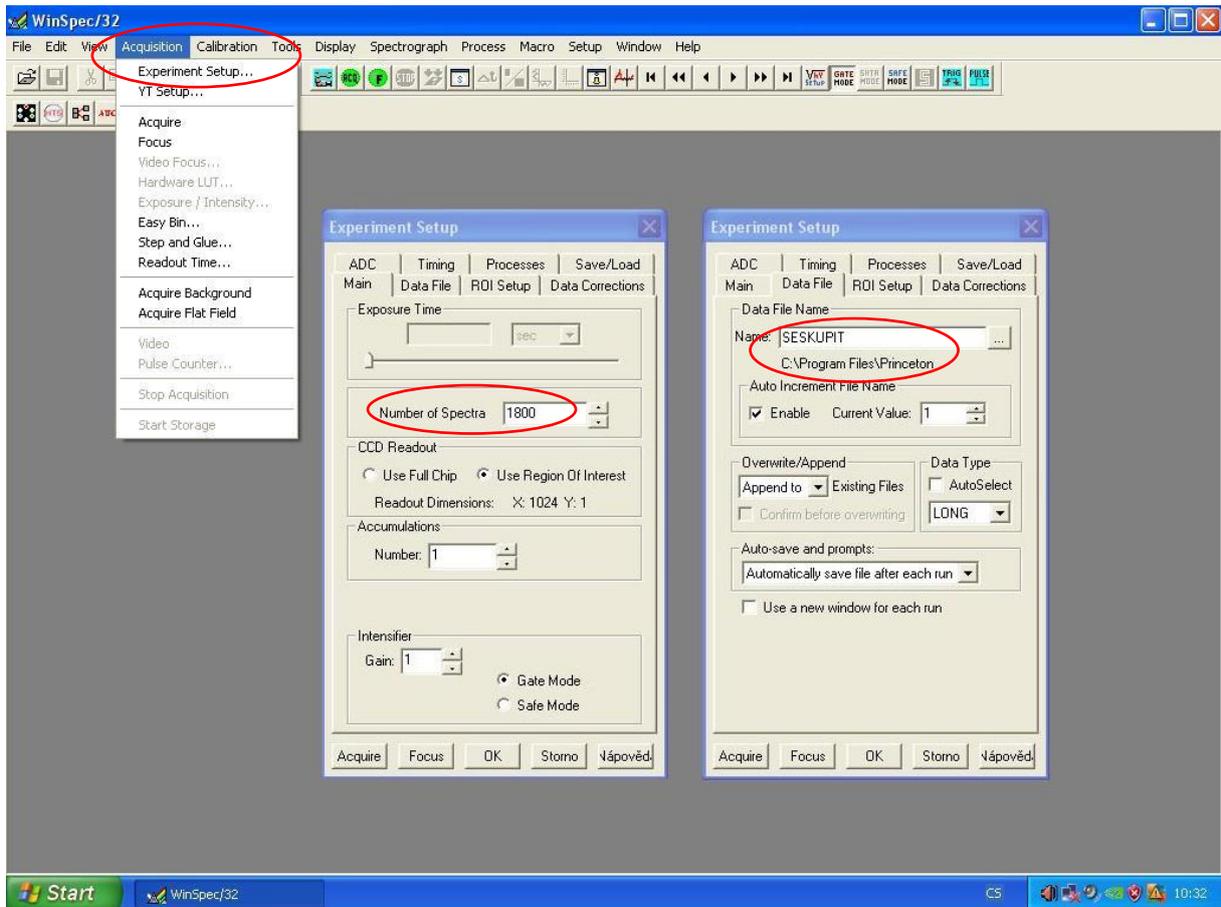
Go to the Dell computer. Set the crater diameter size to 780 μm in the *MEO laser* program. Set the laser energy to 80%.
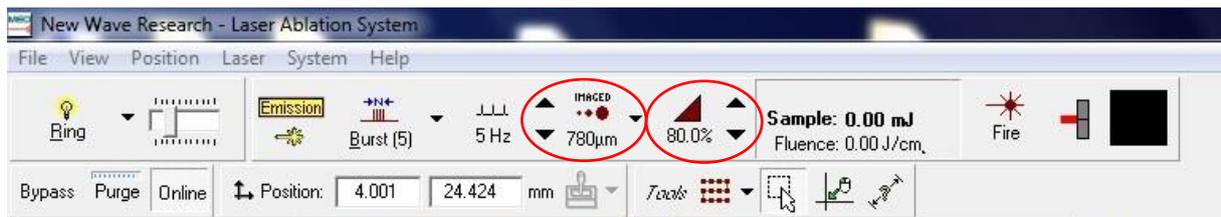
In the *MEO laser* program choose *File → Open Experiment… →* open *rastr 30x30 seskupit.LAE*
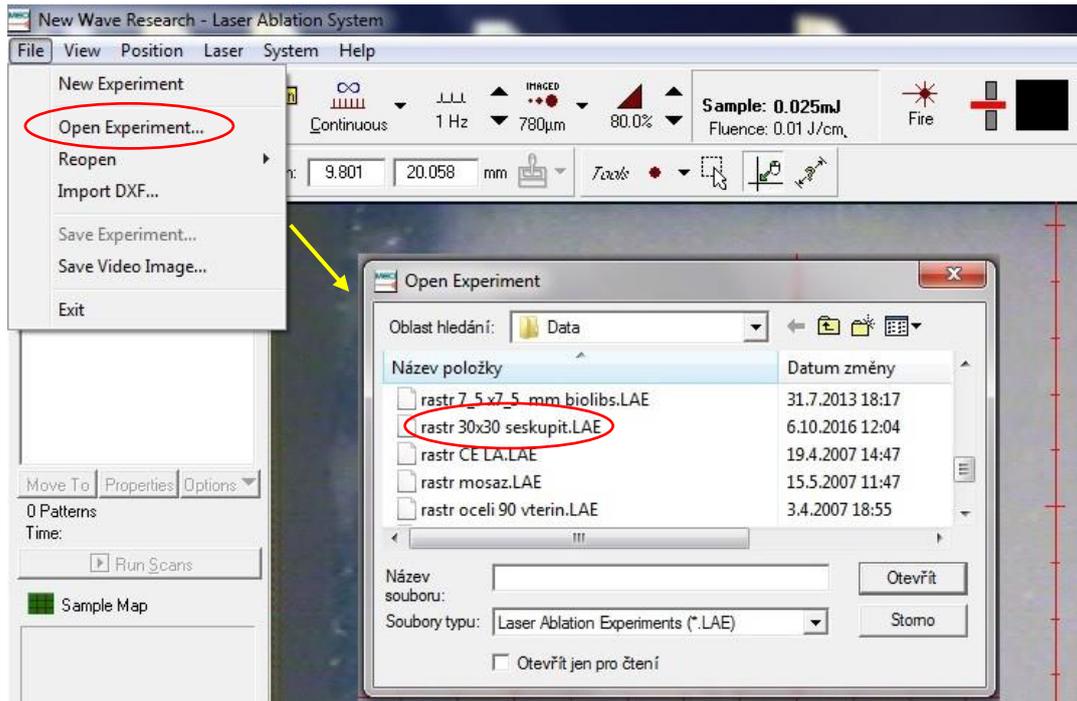
Choose *Properties* and check the parameters setting according to the table
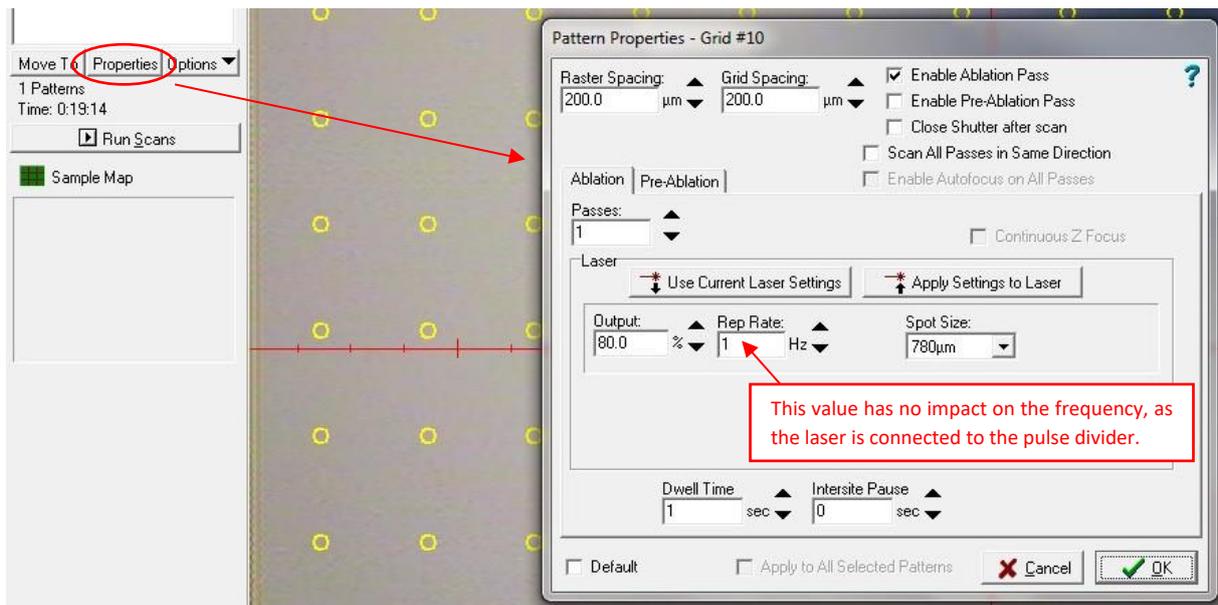
Set the position of the red axes cross according to the picture. Choose *Options → Move Scans to Crosshairs.* The sample should be approximately in the middle of the raster.
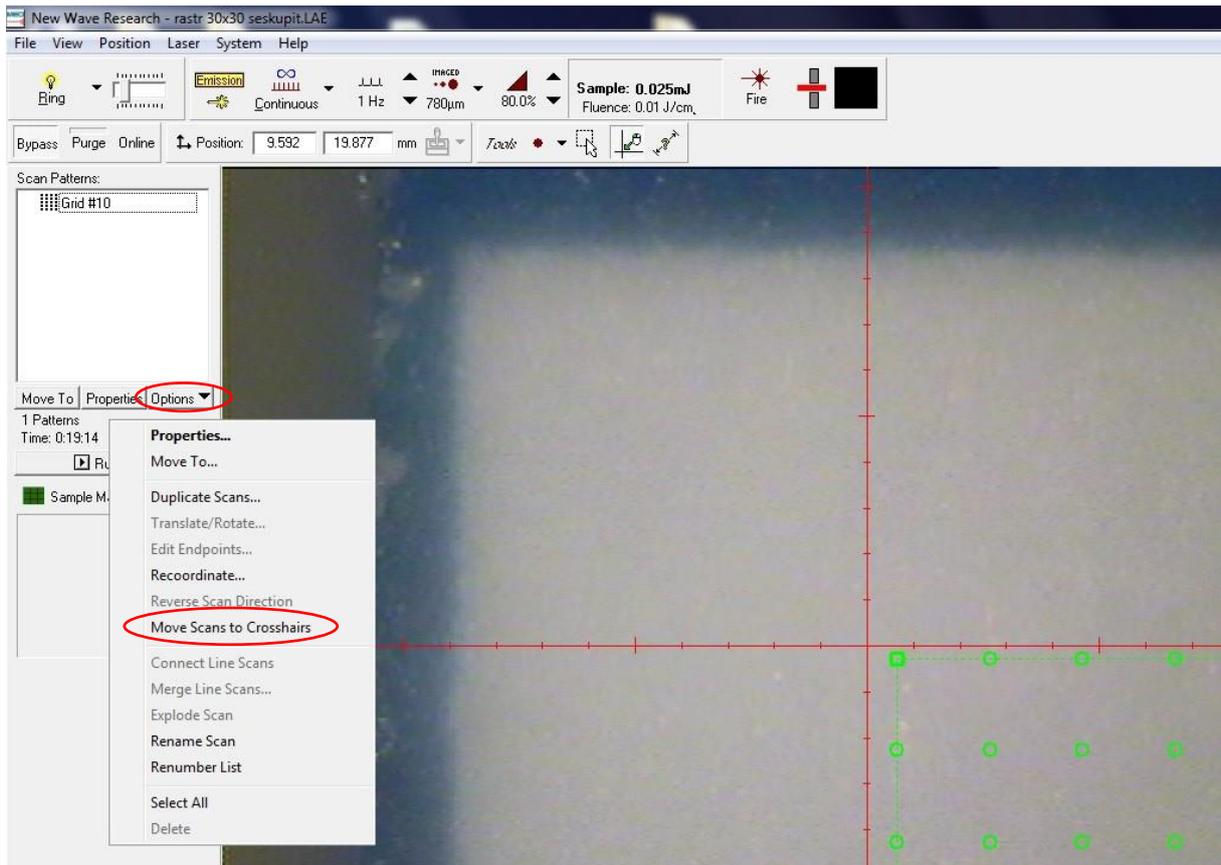
3) Measurements

In the *Winspec* program press the green button *ACQ (Acquire).*
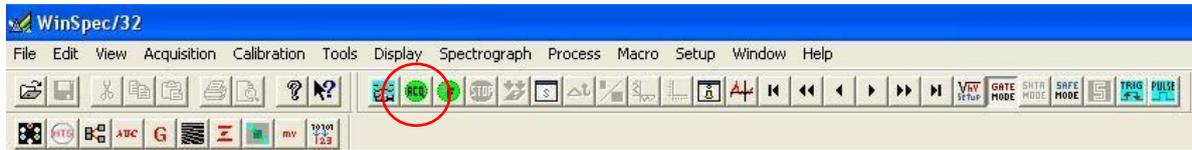
Defocus the sample using the revolving screw
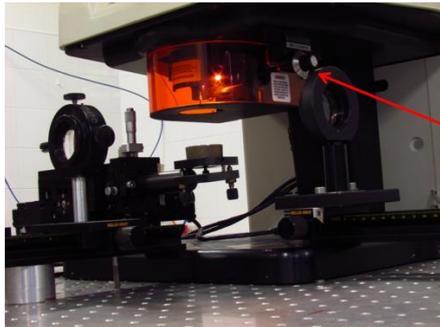
Revolving   screw
for focusing

In the *MEO laser* program choose *Run Scans* → tick *Manually Control Valves* and *Enable Laser During Scans*

!!!ALWAYS PUT ON PROTECTIVE GLASSES BEFORE TURNING THE LASER ON!!!

Choose *Run*
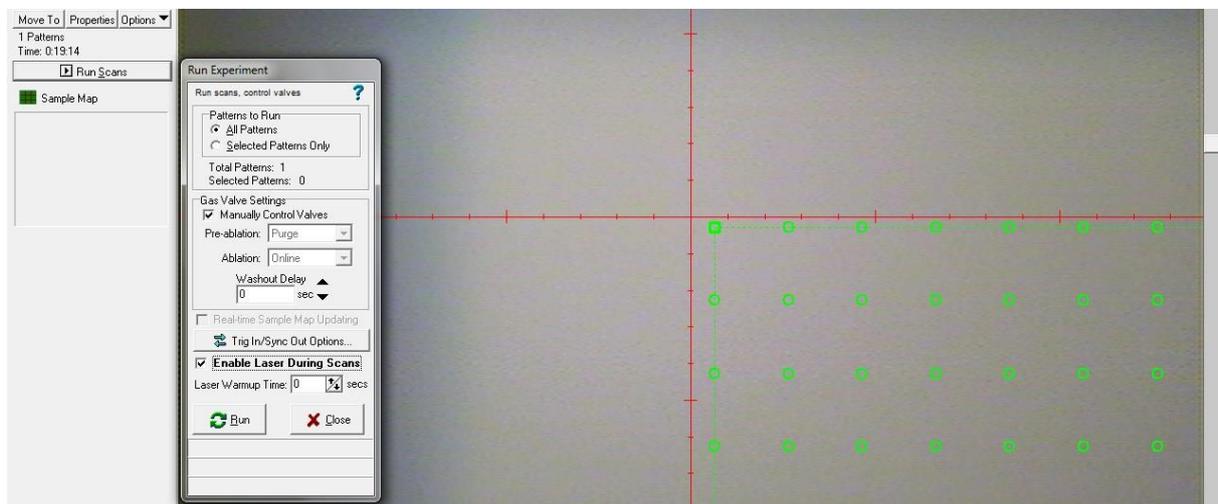
Measurement time ~ 25 minutes
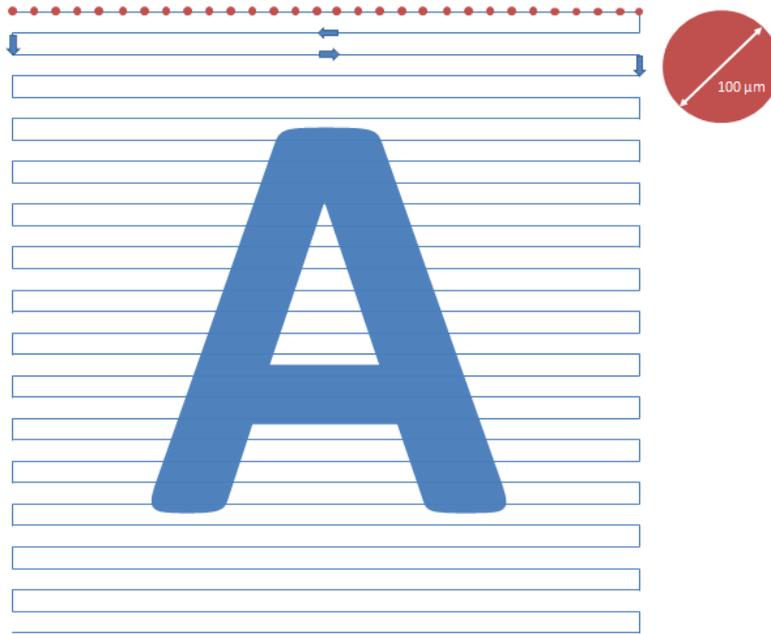
Fig. 5 Raster used for surface mapping – there are 30 points of ca. 100 μm in diameter in each line, arrows show the direction of measurement of individual points

4) Data export
In the *Winspec* program choose the *Tools* tab → *Convert To ASCII*
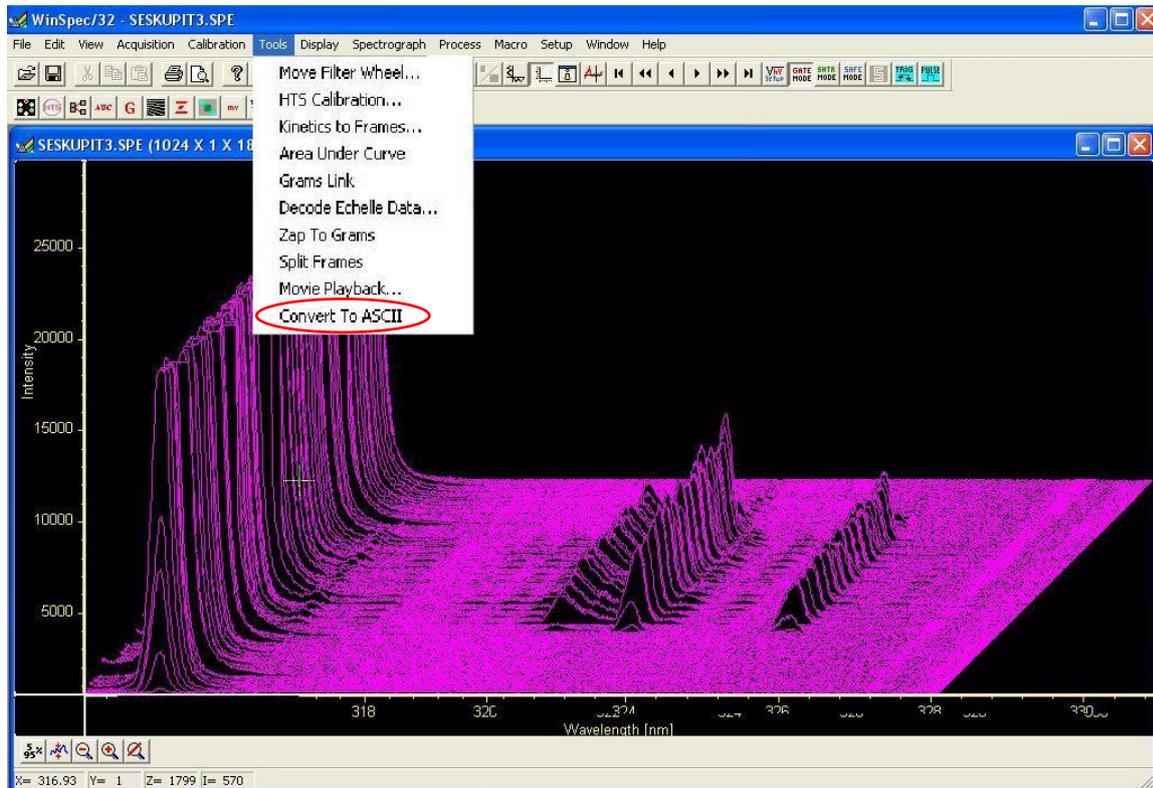
Choose *Choose Files* → Oblast hledání: *C:\Program Files\Princeton Instruments\Winspec\Cviceni SESKUPIT* → Choose and open the last file.
Choose *Choose Output Directory* → *C:\Program Files\Princeton Instruments\Winspec\Cviceni SESKUPIT*
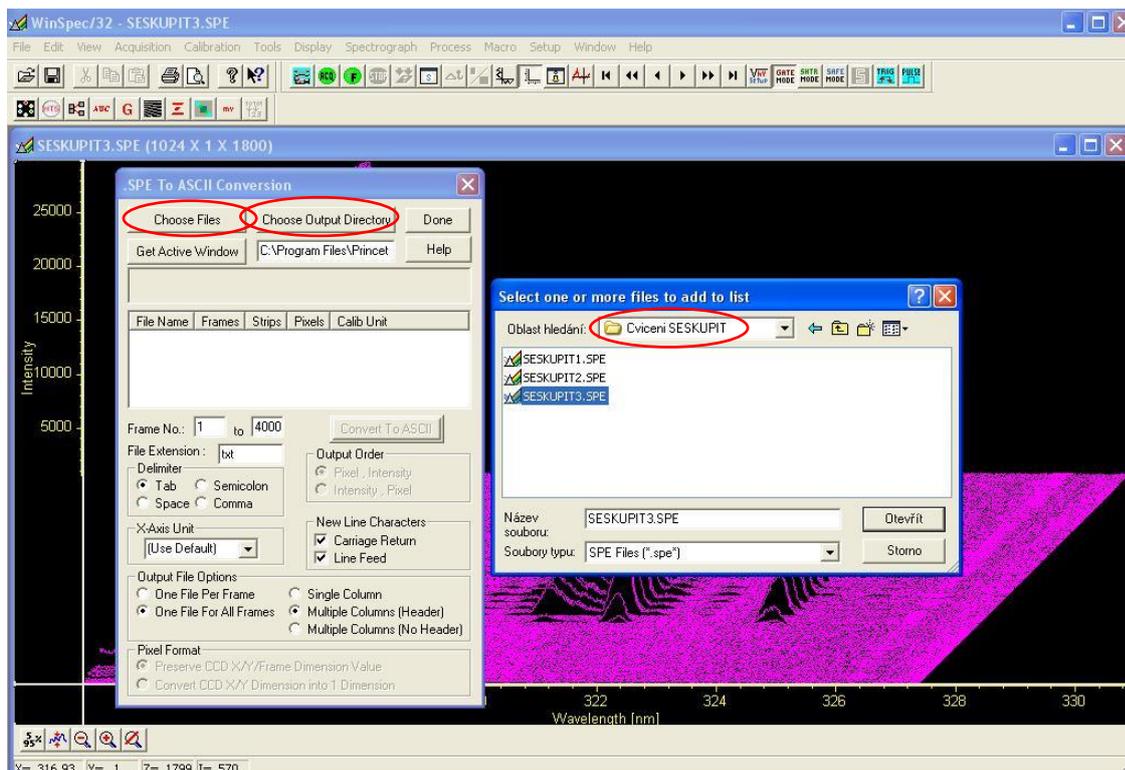
Choose *Convert To ASCII* for selected files. Check the creation of the file *SESKUPIT.txt* in *C:\Program Files\Princeton Instruments\Winspec\Cviceni SESKUPIT*
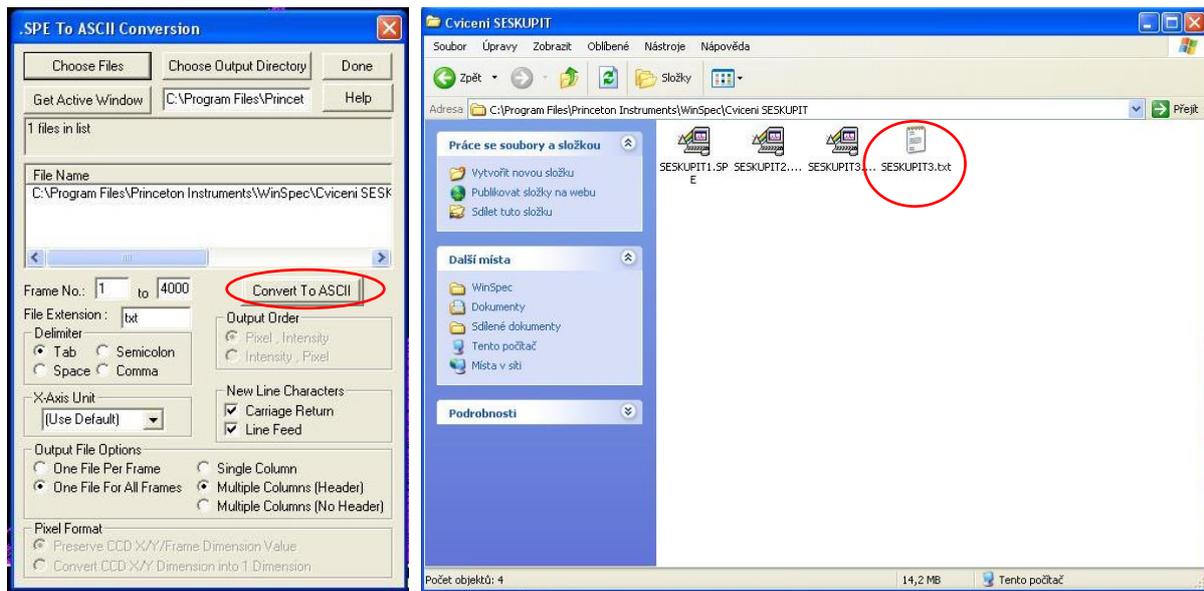
5) Data elaboration

Copy the *SESKUPIT.txt* file to the desktop. Open the *SESKUPIT.xlsx* file on the desktop → choose the sheet *Original data* → choose the tab *Data* → in the first section *Načíst externí data* choose *Z textu* → import the *SESKUPIT.txt* file from the desktop

50
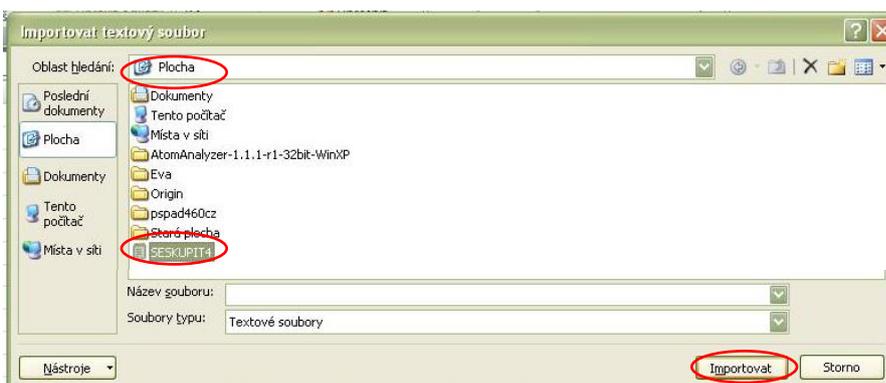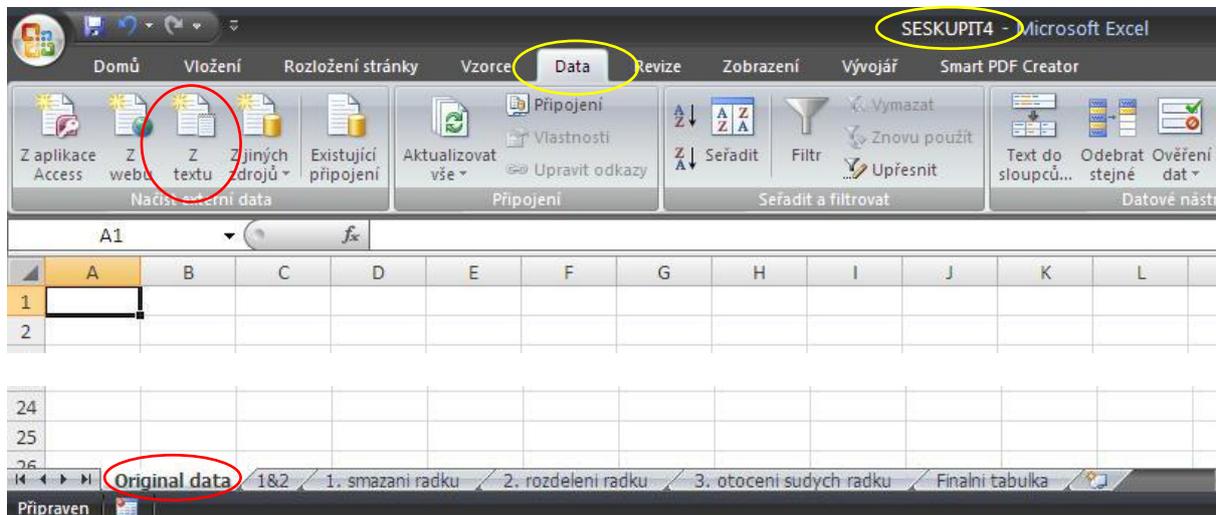
Do not make any changes in *Průvodce importem textu (1/3) and (2/3)* and choose *Další →* in Průvodce importem textu (3/3) choose *Dokončit* → in the table *Importovat data* check the data location and choose *OK*
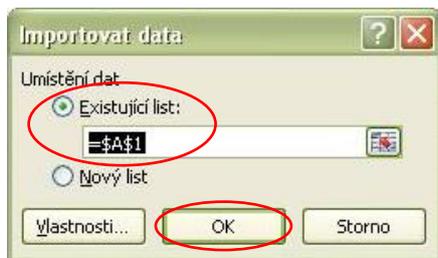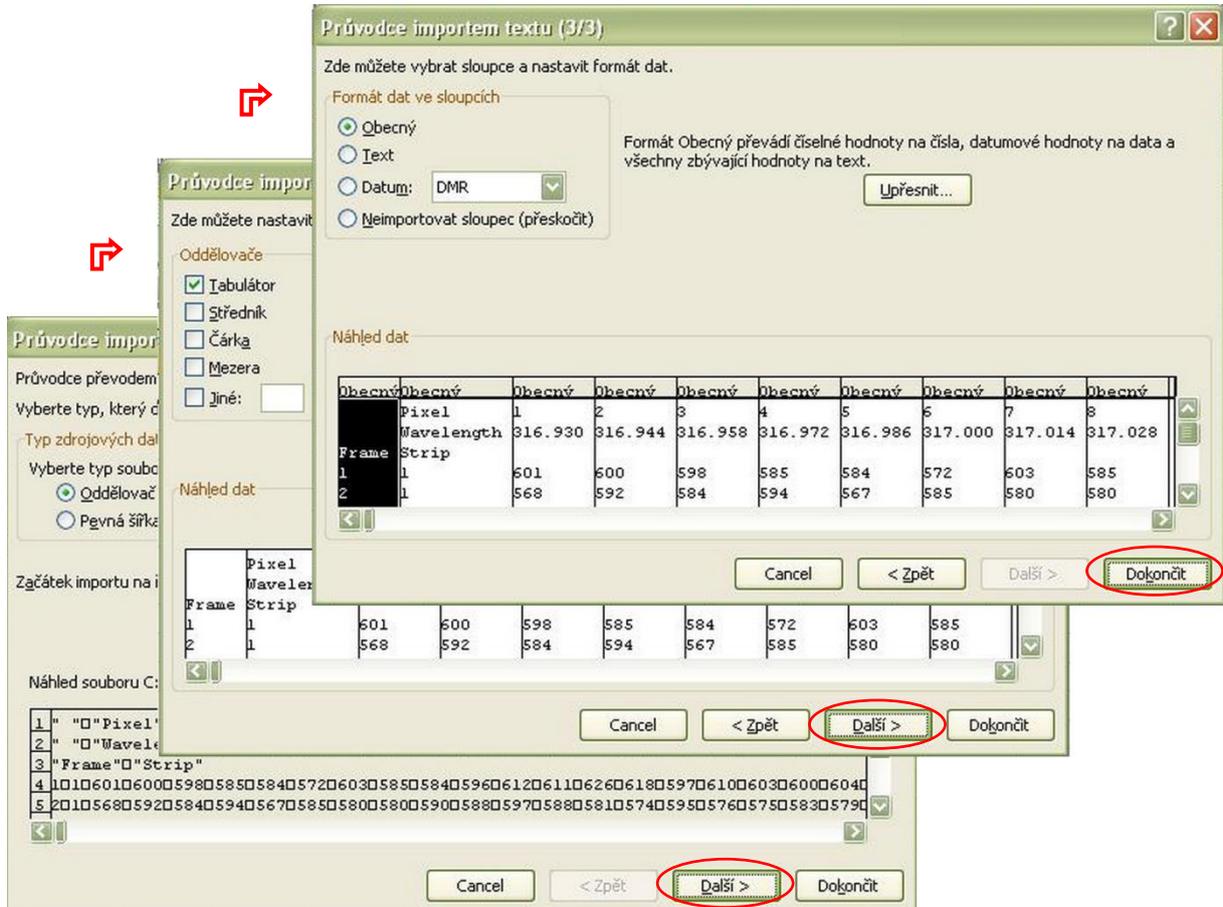
The system adds a vacant even-numbered line with no information to the data file. These even lines need to be removed. Click to A1 box in *Original data* sheet and press *Ctrl+Shift+S*. Data from odd-numbered lines will be copied to *1. smazání řádků*.
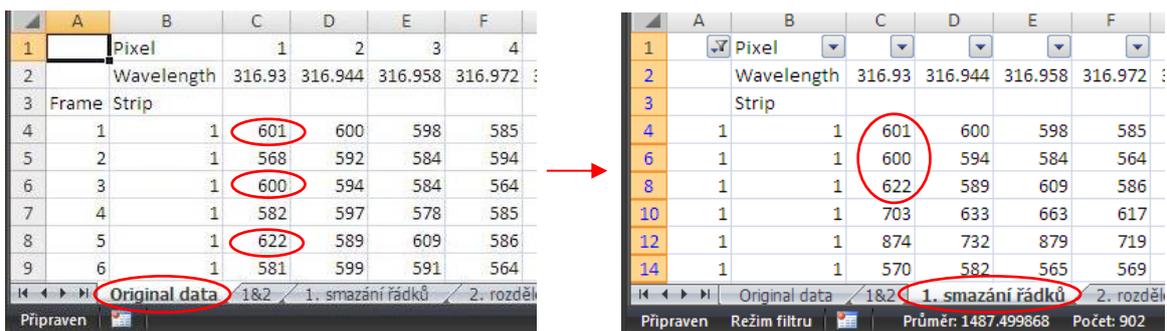
Copy the 324.7 nm column from *1. smazání řádků* with the maximum signal intensity to *2. rozdělení řádků*.

Regarding the course of the raster measurements (Fig. 5), it is necessary to transpose 30 values from the column to the line, while it is necessary to both transpose and reverse the order of the next 30 values (change of the course in the raster in every second line) and repeat until the end.

Click to A3 box and press *Ctrl+Shift+L* for transposition of the odd-numbered lines. Analogously press *Ctrl+Shift+P* for reversal and transposition of the even-numbered lines. Final data for elemental map creation will be displayed in *Finální tabulka*.

	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD
1	620	645	644	625	638	649	619	637	636	643	620	649	629	631	651	610	641	633	631	641	638	655
2	636	649	640	637	611	637	628	658	638	634	647	645	640	651	636	640	653	621	635	639	641	610
3	640	623	638	653	634	652	626	631	632	635	631	656	619	642	643	620	647	631	642	646	637	632
4	649	651	634	632	628	640	619	626	650	646	626	634	636	636	633	627	631	634	626	654	646	652
5	622	617	637	657	628	624	634	652	644	620	630	612	625	642	643	653	639	625	625	640	635	623
6	626	635	617	624	657	635	634	622	637	651	622	634	615	642	627	616	645	629	637	636	634	634
7	618	648	638	642	631	607	646	628	625	629	637	629	651	661	658	649	644	646	634	628	635	663
8	639	629	628	646	625	637	640	652	647	648	632	643	635	637	631	643	652	649	633	626	637	646
9	639	632	639	641	664	1382	3427	3097	1298	651	663	641	676	637	613	624	631	653	616	641	635	638
10	613	665	649	791	1762	7796	7917	7743	7891	879	646	629	650	634	612	657	643	653	660	638	638	660
11	617	637	824	711	2056	8227	7844	7594	7807	1909	858	660	637	646	626	636	644	630	634	637	655	624
12	646	750	935	1390	6710	8780	8240	8246	8585	1102	792	805	785	642	623	631	640	651	632	646	638	654
13	682	719	918	1005	9360	9621	2769	7728	9317	7320	1185	1003	793	671	642	640	645	642	623	640	625	625
14	730	906	1101	4475	10010	8804	1595	5867	8816	8983	1962	1129	888	730	628	631	627	644	636	630	639	646
15	768	817	1068	7420	8604	7535	1036	2234	7645	8856	6483	1214	844	854	664	666	619	648	630	653	608	643
16	784	1172	1927	8001	9894	2169	1663	1345	7381	9974	7783	900	963	839	635	645	636	639	659	631	639	635
17	1018	1111	4459	9078	7244	2635	975	1645	3661	9055	8118	3320	1256	963	741	667	657	657	651	647	647	638
18	1055	1200	7491	9462	10370	6132	10071	9244	8848	9367	9344	3795	1074	820	779	712	675	641	645	639	645	649
19	1025	1751	8950	9100	9487	11208	9236	9887	9991	9765	10018	10650	1676	1153	853	636	646	642	643	638	632	644
20	1313	7645	8115	10367	2447	1717	1903	1778	3635	4501	9364	7256	5277	1138	869	695	669	648	634	626	640	641
21	1464	3806	8229	9337	1836	1794	1874	932	1368	1650	8697	8193	9291	1400	1012	822	651	645	637	648	623	645
22	2353	12084	10883	5941	1687	2079	1425	1423	2101	1264	8150	8933	9705	1537	1042	907	682	664	647	640	638	630
23	1908	2030	1394	2245	1850	1611	1881	2387	1621	1449	1853	1683	1569	1289	1169	769	689	661	637	637	619	641
24	1696	1231	1714	1571	1393	2848	1415	932	1268	1815	1764	1525	1426	986	1155	966	770	700	627	633	647	633
25	1593	1258	1448	1662	1458	1209	1685	1115	1542	1505	1749	1579	958	1070	932	762	706	655	646	641	623	650
26	973	1093	1457	1322	1533	1744	1249	1233	1502	1474	1660	1428	1217	1237	876	851	791	650	653	646	627	649
27	1042	1468	1133	1076	1164	1020	1149	1266	1632	1011	1399	1133	872	720	986	643	637	650	656	648	650	630
28	870	951	687	973	838	1355	1145	1064	1058	907	1041	955	798	924	992	730	724	720	627	643	646	635
29	657	911	957	692	941	1002	950	992	1068	850	960	1035	836	828	806	699	642	640	649	637	647	619
30	775	719	855	862	865	906	940	1010	960	962	998	854	824	769	809	686	718	633	643	669	649	663
31																						
32																						

Mark all values in the table → choose tab *Vložení* → *Grafy* → *Šablony* → choose template according to the picture

Adjust the vertical axis of the elemental map so the maximum value approximately corresponds to the maximum intensities.
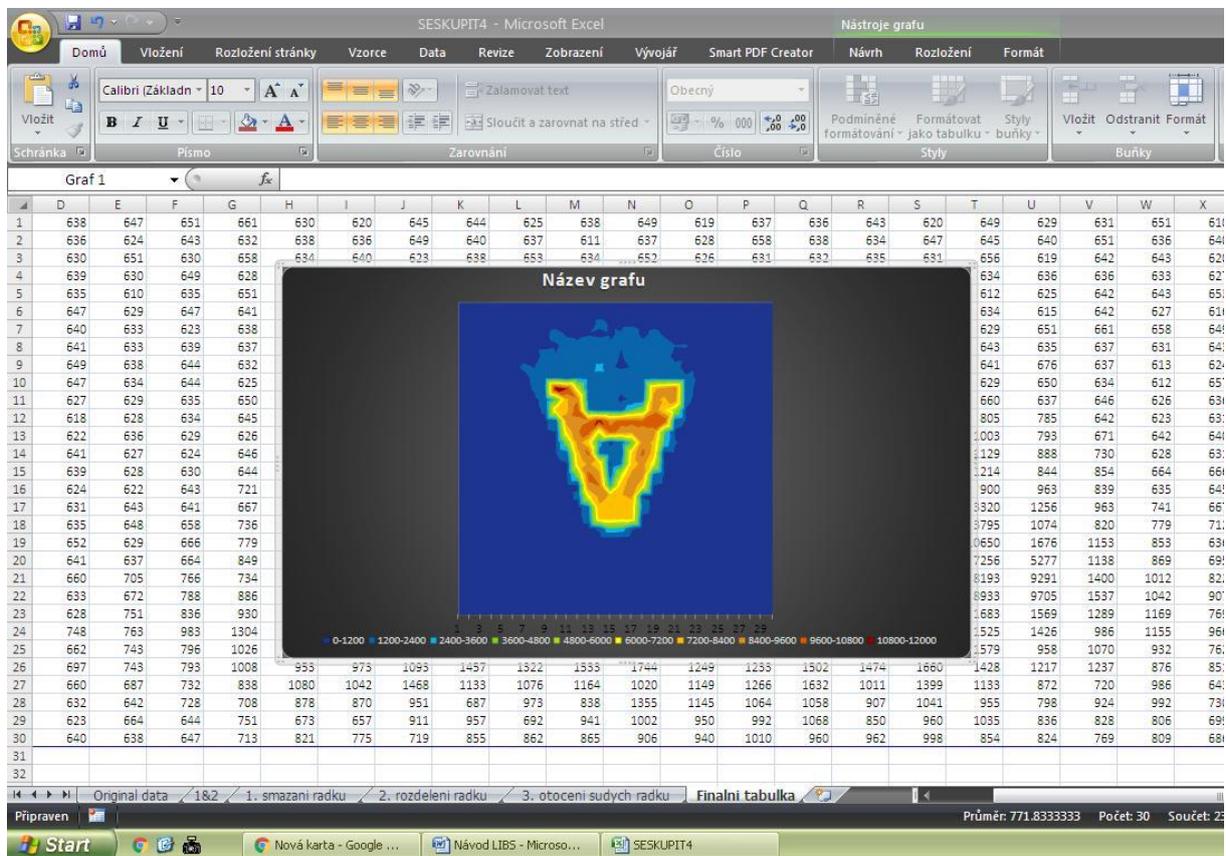
In the *MWSnap* (camera icon on the toolbar) choose *Libovolný úhelník* and mark precisely the area of the map → save the image to the desktop → open the image in the *XnView* program
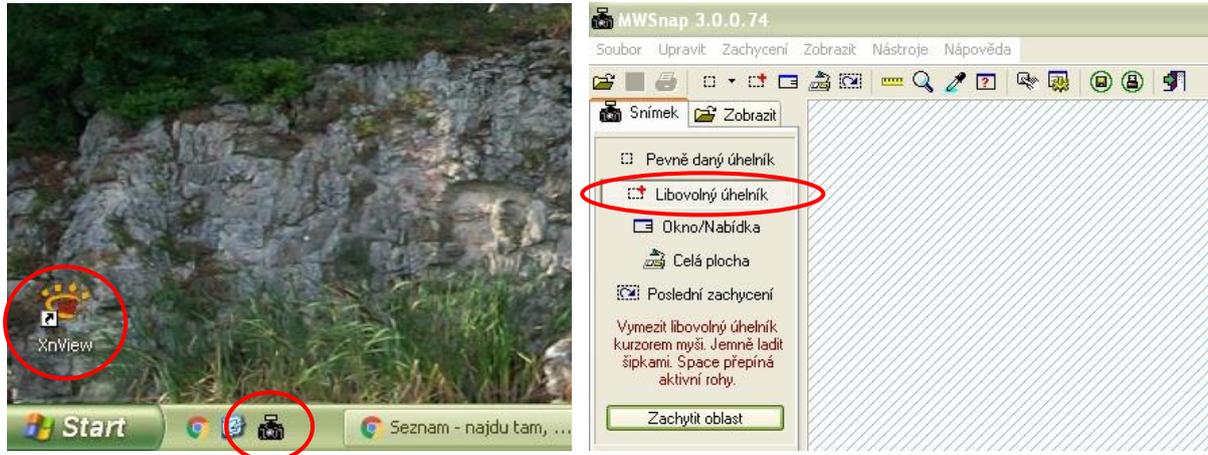

In the *XnView* program choose the tab *Nástroje* → choose *Panorama…*
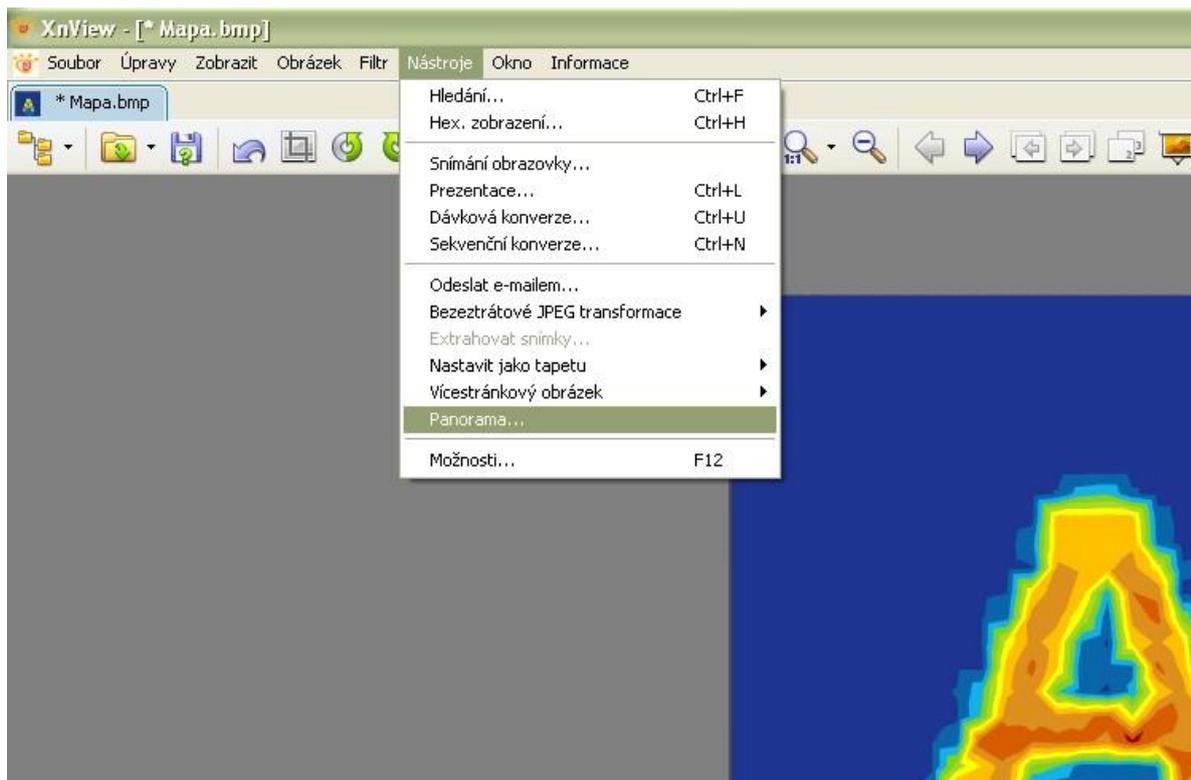
In *Panoramatický obrázek* choose *Přidat…* → in *Zvolte soubory* choose the saved map and choose *Přidat*, then choose *škála 1* and *Přidat* → press OK → back in *Panoramatický obrázek* add selected files, then click on *Vytvořit…*
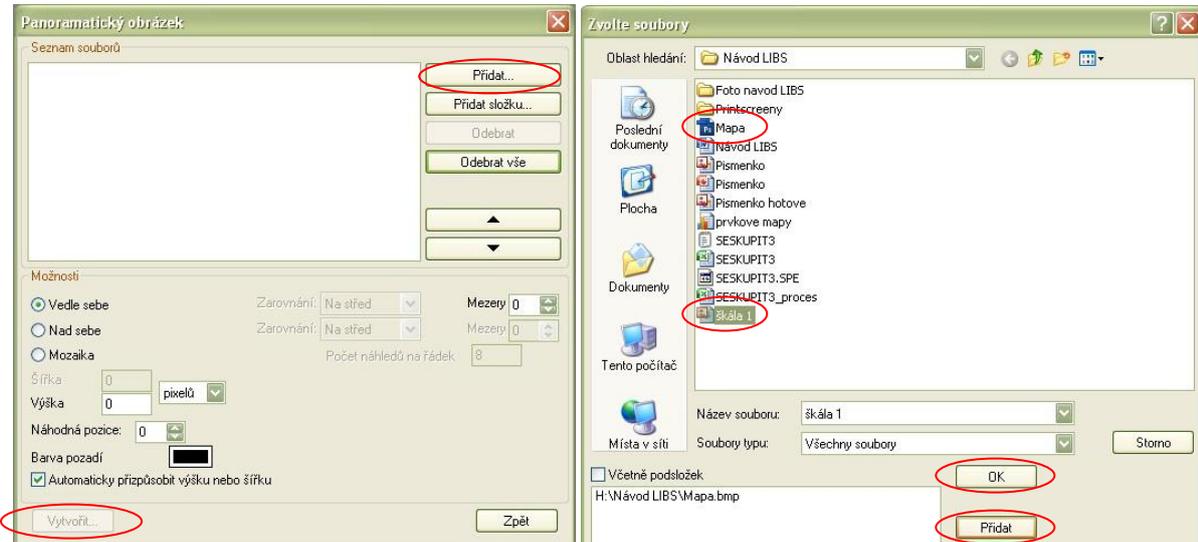
Choose file *Obrázek* → *Přidat text* → add legend accorging to the picture → copy the final map to the lab report
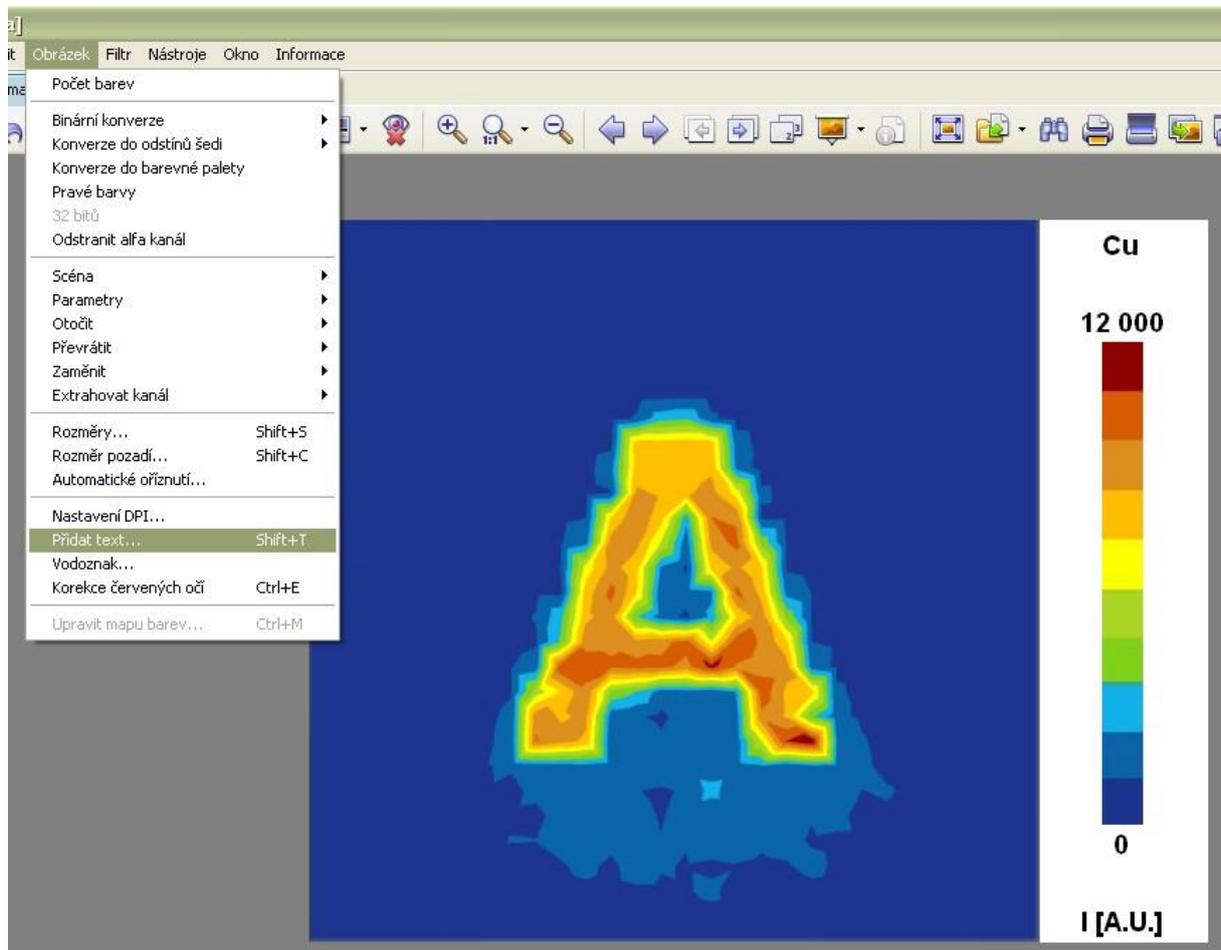
EVALUATION:

Visually compare the elemental maps acquired by both techniques (LIBS a LA-ICP-MS) and describe the differences.

For further comparison of both techniques calculate the detection limit (LOD) and lateral resolution (according to Δw_{rel}).

Limit of detection calculation:

$$LOD = \frac{3 \cdot SD \cdot m_{Cu}}{I_{Cu}}$$

Where:
SD   standard deviation of the background (measured on the plain paper without print)
I_{Cu} average intensity of Cu calculated using at least 5 values in the area of blue print (see LA-ICP-MS instructions)
m_{Cu} weight of Cu in fg on the crater surface of 100 µm in diameter (see Solution analysis instructions)

For the calculation of copper weight per crater surface go as follows:
The content of Cu (m_{celk}) in the whole surface (S_{celk}) of the printed square is related to the crater surface calculated using the laser beam diameter of 100 µm (crater surface is actually bigger than the laser beam diameter, we will, however consider it equal to simplify the calculations).

$$S_p = \pi r^2 = 3{,}14 \cdot 50^2 = 7854 \; \mu m^2$$

$$m_{Cu} = \frac{S_p \cdot m_{celk}}{S_{celk}}$$

Final limit of detection related to the crater surface [fg] will be related to the unit surface [µm^2].

Relative enlargement of the printed line (ΔW_{rel}):

$$\Delta w_{rel} = \frac{\Delta l - l_r}{l_r} \cdot 100$$

Where:
Δl   apparent width of the printed line from the acquired map [mm]
l_r real width of the line (measured in the *MEO laser* program)
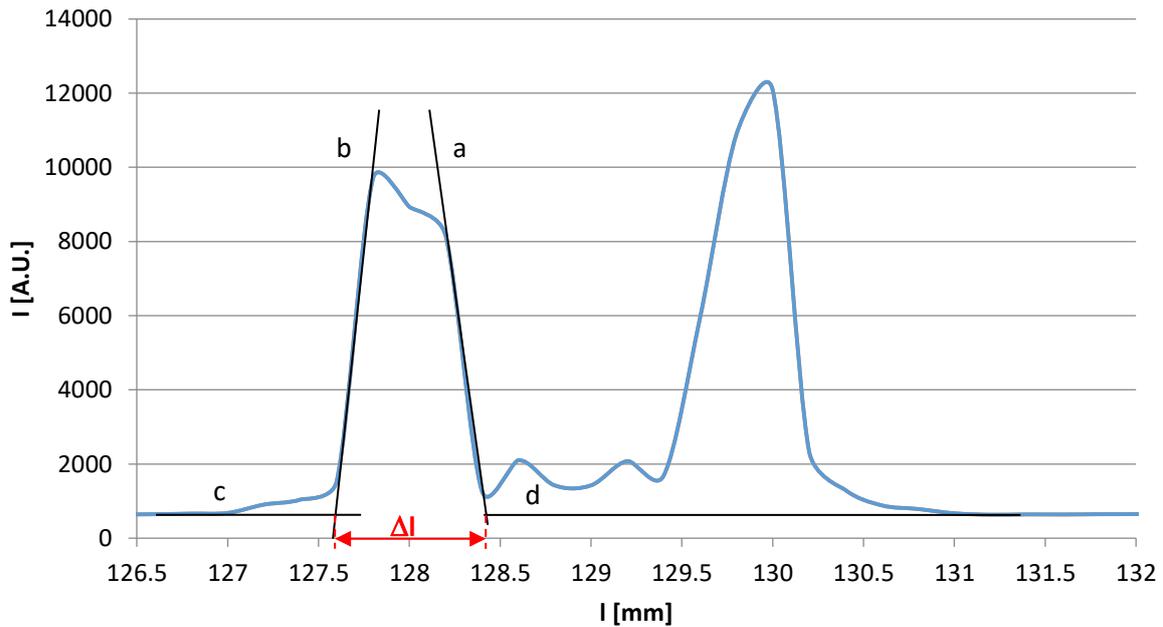
Fig. 6 Apparent width estimation

For calculation of the relative enlargement of the printed line see *LA-ICP-MS imaging* instructions using the graph (Fig. 6).

Lab report will contain:

- Images of Cu distribution in the letter A acquired by LA-ICP-MS and LIBS
- Calculations of relative enlargement of the printed line and limit of detection
- Comparison of the sensitivity and lateral resolution of both techniques based on these parameters
- Comparison of solution analysis (ICP-OES a ICP-MS) and imaging methods (LA-ICP-MS a LIBS) based on LOD related to the unit surface

References:
[1] *What is LIBS?* [online]. [accessed 2016-09-05]. Accessible from: http://www1.uwindsor.ca/people/rehse/15/what-is-libs
[2] Fyzikální princip LIBS [online]. 2014-11-09 [accessed 2016-10-10]. Accessible from: http://libs.fme.vutbr.cz/index.php/teorie/fyzikalni-princip-libs-zaklady