From the Department for Interdisciplinary Dental Medicine and Technology of the
Danube University of Krems, Austria

Effect of the Er,Cr:YSGG laser on Bone Structure under Controlled Experimental Conditions.

A Histological Study

Master's thesis

for achievement of a degree as
"Master of Science for Oral Surgery" (M.Sc.)

submitted by

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Examiner: Prof. Dr. Dr. Rainer Schmelzeisen

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1. Introduction

The trials for the bone studies presented here were already concluded in 2004. In order to carry out these studies under controlled conditions, a test assembly was designed and registered as a utility model[1]. This test assembly was explained comprehensively in my master's thesis to achieve an M.Sc. in implantology under the title of "Design of a standard test assembly for controlled laser studies on tissue".

The subject of the experiment at the time was untreated human bone exposed to the Er,Cr:YSGG laser of the company Biolase[2]. This laser is an opto-mechanical disruptive laser.

It was only possible to evaluate the histology at a later date. The work cited above dealt only briefly with the macroscopic results for the preparations. This is still the only work which deals with the histology of genuine human bone after treatment with the Er,Cr:YSGG. My last lecture to the World Clinical Laser Institute Supersymposium 2007 in Los Angeles again made this clear.

This work deals with the macroscopic and microscopic results for the laser-treated bone preparations.

At present – and this is well known in the meantime – only water spray cooling erbium lasers of class Er,Cr:YSGG [Lit. 1, 2, 3, 4, 5, 6, 7, 10, 14, 15, 16, 17, 19, 20, 21, 23, 24, 25, 30, 35, 36, 38] and Er:YAG [Lit. 2, 9, 10, 13, 14, 18, 19, 22, 27, 30, 33, 37] are used here or are actually exclusively indicated.

This study had the aim, chosen by me, of verifying the success of the laser application for human maxillary and mandibular bones. These trials were drawn up in accordance with strict scientific parameters and not according to random application studies which are already well known. My study tells the practitioner (including myself) that the Er,Cr:YSGG-Lasers can be used safely for processing bone provided certain simple parameters are observed.

[1] Registered German utility model No. 20 2005 000 844.3

[2] Biolase Technology Inc., Irvine/Cal., USA

It is not the task of this thesis to explain the diverse applications on bone with regard to ostitides, peri-implantides, preparations for implantation bed recovery, implant preparation and other laser-indicated therapies. It is only aimed at underlining their possibilities and verifying them as methods [Lit. 1, 3, 6, 11, 15, 17, 19, 20, 21, 30, 35, 36].

There is nothing in dentistry that you could not use lasers for! But we should search for new and more comfortable treatments for our patients. That is a challenge for the innovatively-minded dentist.

2. Material and Methods

2.1 Removal of unfixed human bone

To carry out the laser study, bone was taken from the following areas:

- mandible (premolar to molar region)
- mandible interforaminal region
- sinus maxillaris (regio tooth 3 to 5)

The brisk removal took place directly on the unfixed total preparation (time of death was between 2 and 4 days before hand) using a Lindemann grinder with cooling. The preparations removed were brought in a sealed, humid chamber (cellulose dampened with 0.9 % NaCl), cooled with ice water, to the trial location and processed within 6 hours with the laser test assembly at a room temperature of 24° C.

2.2 Test assembly 1

2.2.1 Preliminary considerations for the test assembly

The plan was to carry out a controlled study while maintaining a defined distance of the laser processing tip over the bone to be processed.

2.2.2 Inspection of the test assembly

As suspected, an exact distance cannot be maintained in a non-contact treatment of the bone by the laser. Equally, speed and path are neither reproducible nor exactly allocable [Lit. 3, 6, 15, 21, 27, 33, 35, 36]. This is why test assembly 2 was developed.

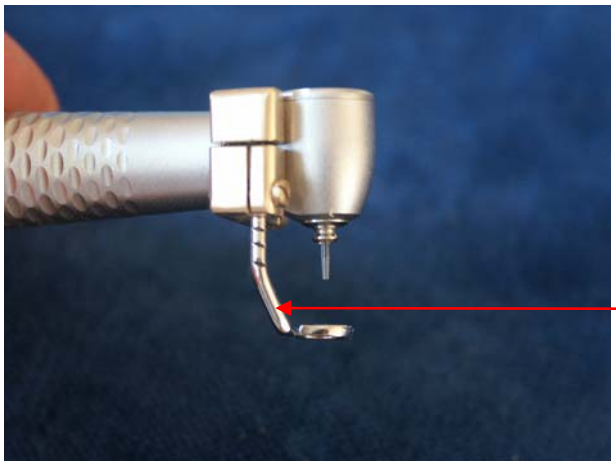


Fig. 1: Hand piece, detail view including laser tip with visible calibration

2.3 Considerations regarding test assembly 2

It seemed a good idea to select the test assembly in such a way that an exact distance as well as a reproducible time-path relation for controlled time exposure studies of laser on different types of tissue can be described. To the present day no such test assembly can be found in the literature.

This second Test Assembly will be published soon under “Construction of a Standard Test Assembly for Controlled Laser Studies in Tissues – Preliminary Study on Human Bone Material”. Authors: Franziska Beer and Harald Passow.

2.4 Test assembly 2

In order to include the factor “time” in a controlled manner, the following conditions had to be fulfilled:

2.4.1 Technical conditions

a) The preparation must be able to be moved on a tray in X and Y axis.
b) The control cannot be accomplished ‘by hand’. It must take place by means of stepping motors and be absolutely reproducible. The movements of the workbench and the drive by stepping motors must execute an even movement of the preparation, i.e. that respective feed motion over the x- and/or y-axis must run harmoniously and without jerk.
c) A computer must be used to control the laser beam, which transfers variable control programs to the step motors.
d) Supplementing water and air supply should be possible as an extension of the experimental assembly.
e) Suction of the resulting liquids must be possible.
f) The mounting of different hand pieces of various lasers for comparative investigations should be easy to apply.
g) The control programs must be able to copy temporal hand guidance.
h) A step motor control should be later possible to vary the ups and downs of the laser hand piece over the object (bone, tooth, etc.).

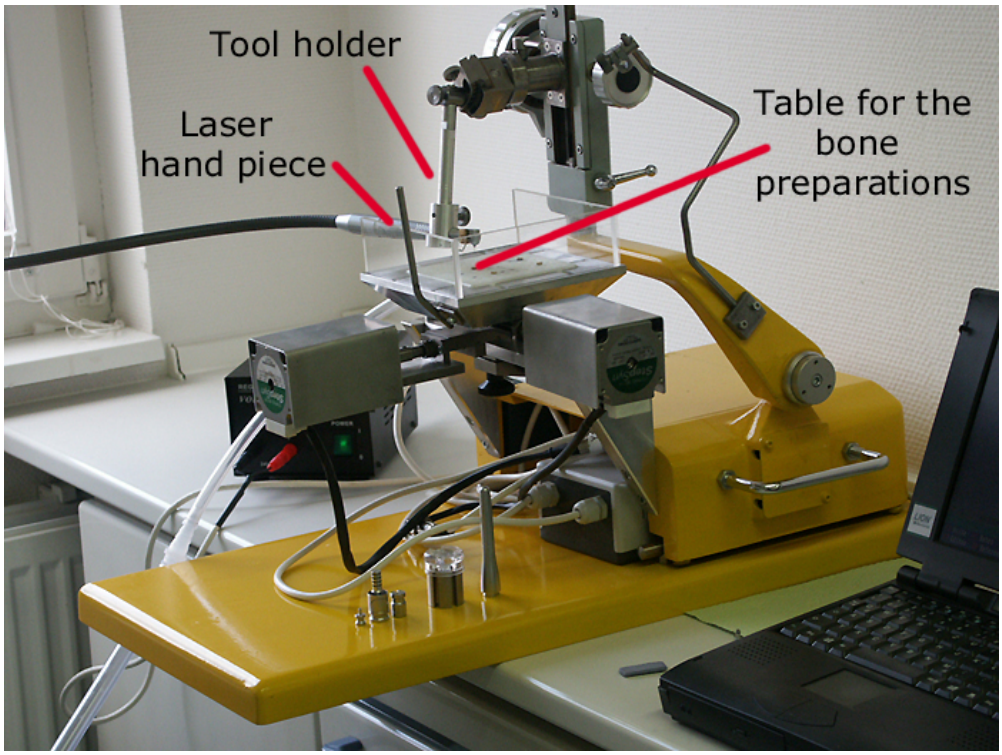
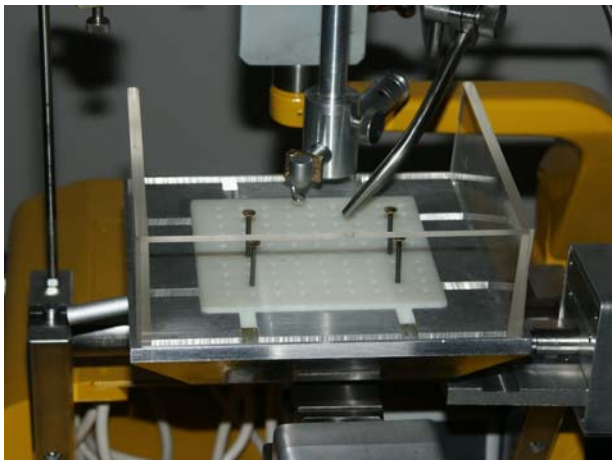


Fig. 2: Representation of the test assembly

This idea and development is the subject matter of registered German utility model No. 20 2005 000 844.3



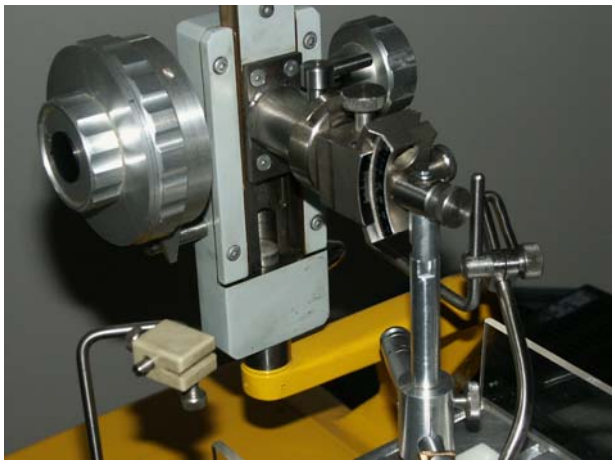


Fig. 3 a,b, c: Details of experimental assembly

Fig. 4: Complete experimental assembly

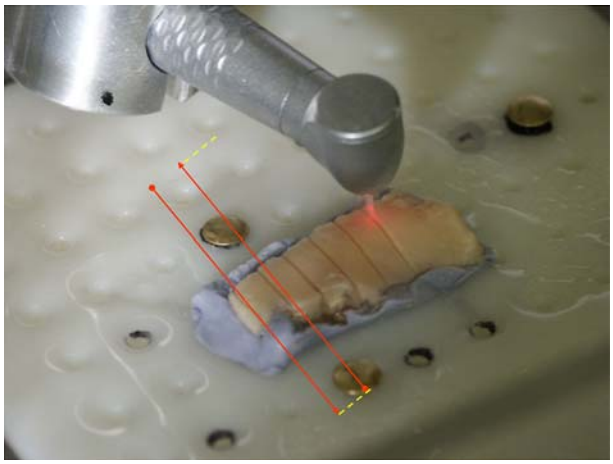


Fig. 5: Laser in working position over a bone

2.4.2 Requirements for possible applications

a) The equipment is designed in such a way that not only hard-tissue, but also soft tissue can be examined.
b) Experiments can also be carried out on the decontamination of infected implants. (The Waterlase does not cause heat in implants.)
c) Investigation for the weakening of the Compacta as pre-treatment at the spot of the pilot drilling (easy and safe guidance for arranged implant bed preparation).
d) Decontamination studies would also be possible on infected root surfaces of natural teeth and pocket epithelium (discipline: periodontology).

2.4.3 Dimension of the tissue material to be examined

Since human tissue is available due to the withdrawal areas only to a limited extent, it had to be possible to treat tissue parts of 2 x 2 cm several times.

2.5 The following laser was used for the experiments

Manufacturers:	Biolase Technology Inc., Irvine/Cal., USA
Norm:	Biolase Millennium
Laser class:	IV
Media:	HE, CR: Y S G G (Erbium, Chromium, Yttrium, Scandium, Gallium Garnet)
Wave length:	2780 nm
Frequency:	20 c.p.s.
Performance accuracy:	20 %
Pulse energy:	0 … 300 mJ
Pulse duration:	140 … 150 µs
Specimen angle:	Default: 90°
Tip Size:	200 … 750 µm
Ray enlargement:	8 %
Mode:	Multi-mode
Pilot ray:	Laser diode, laser class I, 655 nm
Hazard distance:	5 cm (NOHD)

2.5.1 Accessories used for laser processing

Trial handpiece: Angle handpiece 90 % Standard

Sapphire tips: Tip G 6 or C3

Tip type	Diameter (µm)	Calibration Factor	Lengths (mm)	Tissue Types
MG6	600	1.00	6	Enamel Dentin Bone Soft Tissue
C3	300 x 1200	1.00	9	

Distance to tissue: 3 mm (±15 %)

Angle position: 25°

(The angle position was set in test assembly 2 at 25°. Due to a certain degree of unevenness of the bone surface, a deviation of ±5 % must also be taken into account.)

Setting: Supply of distilled water / compressed air (For variables see test protocols in 3.1.1, 3.1.3 and 3.1.5)

Power settings: 3.5 and 5 Watt

The following images illustrate the different wave lengths most often used for lasers in dentistry.

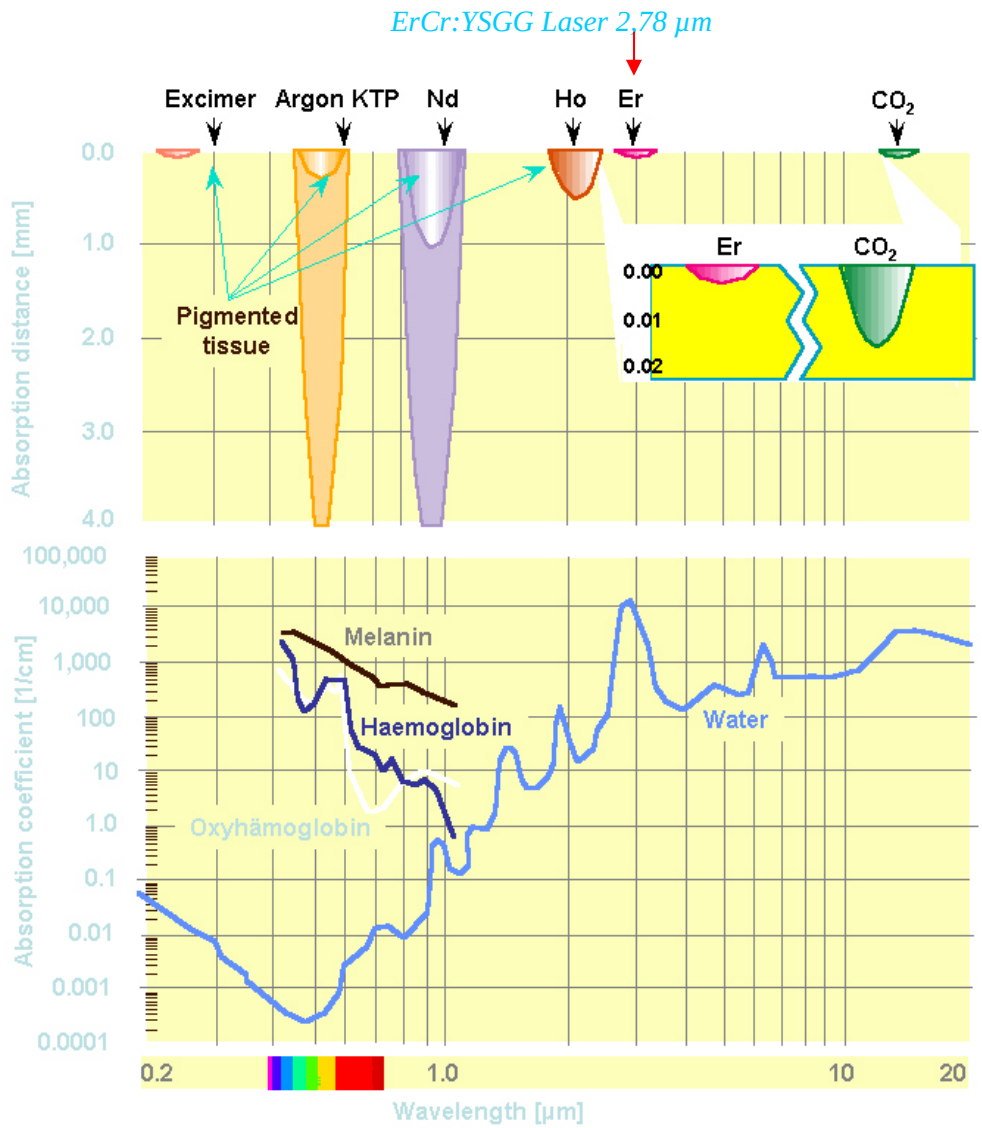


Fig. 6: Comparison of physical tissue parameters and different lasers

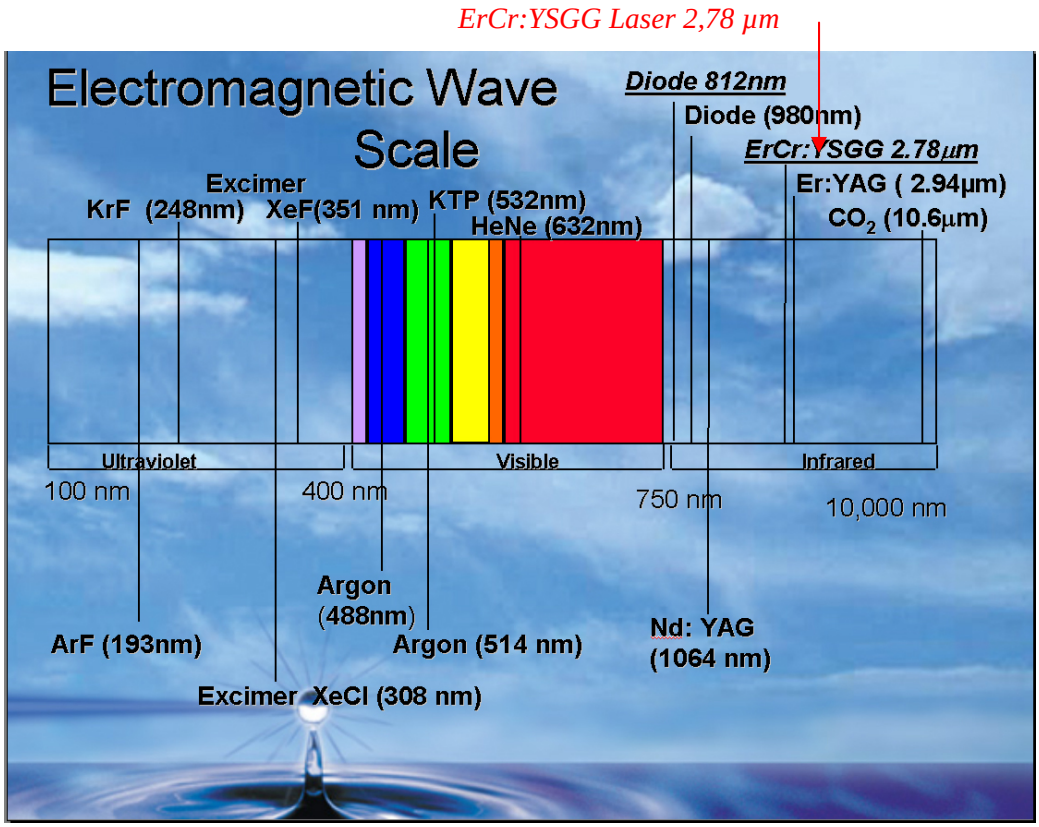


Fig. 7: Electromagnetic Wave Scale

2.5.2 Reference setting

The precise reference setting of the laser handpiece was carried out by the integrated target beam of the laser (laser diode, laser class1, 635 mm) by means of a control program.

2.6 Execution of the experiment

2.6.1 Preparation of the donor bone for embedding on the preparation table

As the bone recovered has an uneven surface, the spongy side and the lateral compacta areas were prepared with a grinder and plenty of cooling with 0.9 % NaCl.

2.6.2 Alignment of the laser handpiece

First the processing angle of the laser on the height support was aligned with the mounted angle setting device and fixed over the reference point. The working height above the bone was only set after the bone was embedded by running the reference program. The bone to be processed was embedded in such a way that the reference point was at the height of the first preparation cut (Fig. 8). Aligned in this way, the first processing step could be carried out starting from the reference point in the respective control job over the preparation.

2.6.3 Addition of air and distilled water

The air quantity of the spray (adjustment of H_2O/air) could not be established.

Calculated in terms of the path traveled of 1 mm/second, the quantity of water applied to the bone at the setting 65/65 corresponded to 0.67 ml/sec. The actual water output was measured at the handpiece. It amounted to 40 ml/min.

However, this value cannot be linearly correlated with the values set at the panel (Table 1). In addition to this, a number of comparative measurements showed deviations of 5 to 10 %.

2.6.4 H_2O setting at the panel of the laser device

Value	Setting for H_2O at the laser device	Measured quantity at the handpiece in ml/min	Actual output in %	Deviation over a number of measurements	Actual water output calculated in ml/sec
A	100 %	72	100 %	5 – 10 %	1.2
B	80 %	66	91 %	5 – 10 %	1.1
C	60 %	46	64 %	5 – 10 %	0.77
D	40 %	36	50 %	5 – 10 %	0.6
E	20 %	30	41 %	5 – 10 %	0.5
F	10 %	22	30 %	5 – 10 %	0.37

Table 1: Comparison: H_2O setting values at the laser device panel and actually measured values.

2.6.5 Embedding of the bone for processing

It was first planned to fix the bone to the perforated Teflon plate using special holding elements. In the pre-trial stage this proved too difficult (no picture) and time-consuming. As a pressed-on additive cross-linked silicon which was then cut to size after setting was used to establish the reference (Fig. 5), this material was also used to embed the bone material.

A silicon molding paste with the property of “slow setting” was prepared and pressed onto the perforated plate within the reference work field. The bone was pressed into this embedment mass and aligned in the horizontal and vertical planes. After setting, the superfluous silicon material was cut to the required size using a scalpel.

Removing the bone after the trial did not pose any problems. .

2.6.6 Selection of the processing program and execution

The respective "job" was selected and the work program for the corresponding test started.

Data on the PCNC[3] runs	
All jobs start at the marking at the top left and return to the same place at the end!	
Reference job	Path from the marking point at the top left to the bottom right, the top right, bottom left and then back to the marking point at the top left. *(Automatically loaded to set the laser when "laser" is entered in MS-DOS mode, but not started.)*
The following three 3 PCNC runs were used for this work:	
24mm_um3.job	path 24 mm horizontally in 24, 48, 72 seconds; each shifted vertically by 3 mm in 30 seconds
24mm_um2.job	path 24 mm horizontally in 24, 48, 72 seconds; each shifted vertically by 2 mm in 30 seconds.
24mm_um4.job	Path 24 mm horizontally in 48, 72, 96, 120, 144, 168 seconds; each shifted vertically by 4 mm in 30 seconds.
Square1.job	Path 10 mm horizontally and 8 mm vertically to the start of the object and then: path: 10 mm horizontally in 10 seconds, 10 mm vertical in 10 seconds, 10 mm horizontally in 10 seconds, 10 mm horizontal in 10 seconds;
Square2.job	Path 10 mm horizontally und 8 mm vertically to the start of the object and then: path: 10 mm horizontally in 15 seconds, 10 mm vertically in 15 seconds, 10 mm horizontally in 15 seconds, 10 mm vertically in 15 seconds;
Square3.job	Path 10 mm horizontally und 8 mm vertically to the start of the object and then: path: 10 mm horizontally in 20 seconds, 10 mm vertically in 20 seconds, 10 mm horizontally in 20 seconds, 10 mm vertically in 20 seconds;

Table 2: Data on the PCNC runs

[3] CNC = Computerized Numerical Control
NC = Numerical Control

The reference job was established on the basis of the possible movement lengths of the cross support and marked on the preparation table. The fixed step motors are connected by the respective axles directly with the spindles of the cross support. The use of gimbal connections was rejected (step clearance of gimbal-type shafts). The spindle lift of the cross support amounts to 0.8 mm/360° rotation. The step motors are actuated by a control card which in turn receives its instructions via the control program of the computer.

The power is supplied via a direct current power supply unit at 24 Volts.

The step motors operate at a frequency of 5 cycles/mm. The spindle lift of the x and y axes is identical. A 360° rotation moves the preparation table by 0.8 mm at a time in the horizontal or vertical direction.

I set 1 mm/second as the smallest movement unit (feed movement of the preparation table via the coordinates). Under this time no laser can have any useful or measurable effect within in dentistry applications.

The limit range of 1 mm / 7 seconds was established as the maximum movement. The paths of the x and y axis of the tissue to be moved were selected in such a way that the different laser parameters can be easily varied between operations (Table 2).

2.6.7 Mode of operation of the preparation table

The PCNC run “24 mm_um3.job” (Table 2) was taken as an example for the description of the executed processing programs

The table with the preparation moves in a horizontal direction over a path of 24 mm at a speed of 1 mm / second, then changes the processing direction to the vertical by 3 mm in 30 seconds. Then the table runs the next job on a parallel axis over 24 mm at a speed of 1 mm / 2 seconds, before changing to the vertical again by 3 mm in 30 seconds. After the program has run (in this example this means three runs at different speeds) the table returns automatically to the reference point.

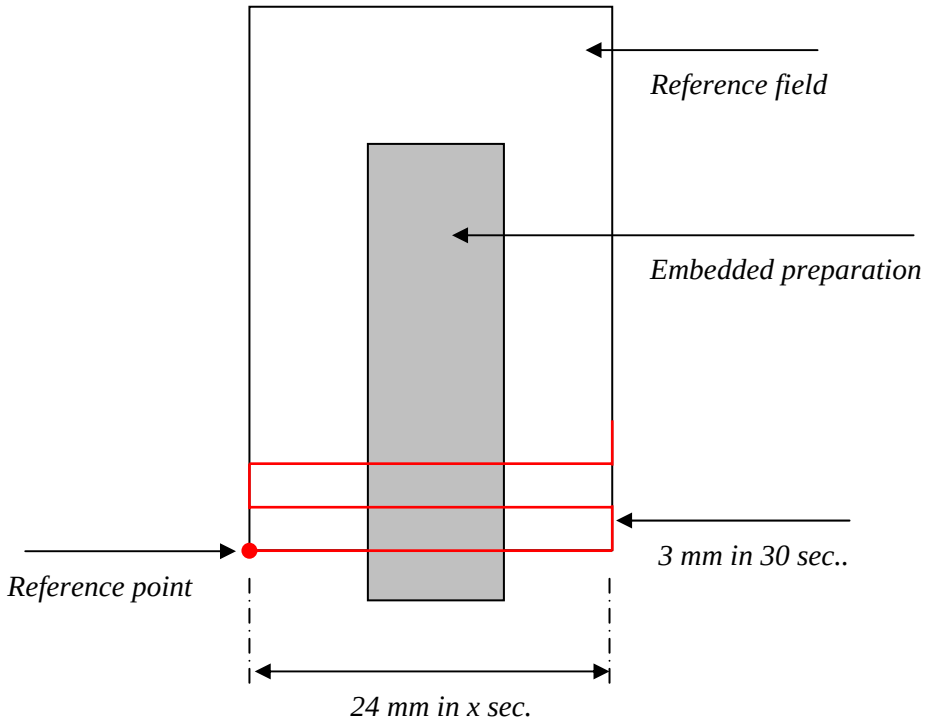


Fig. 8: Diagram of the PCNC run of a processing program

Due to the control programs the exact same job can be run twice on the same preparation (see example in the Results, 3.4.1.5 preparation B1). This example shows the exact reproducibility of the control program.

During the trial run the laser was only switched on and off over the position of the preparation with the aid of a foot switch.

A strong vacuum (dental treatment unit) was used to draw off any moisture from the preparation in the direction of the preparation table).

2.6.8 Preparatory work for the histological section preparation

After the end of each test, the bone was released from the embedding material and the preparation transferred immediately to the fixing solution:

For the preparation series 1/0, 2/0, 3/0, 4/0 and A1, A2, A3, A4, a fixing solution of 8 % formaldehyde, 70 % undenatured alcohol was used. For the preparation series B1 and B2, a fixing solution of 4 % formaldehyde in 70 % undenatured alcohol was used.

Before histological processing, the hard tissue parts were documented by camera.

2.6.8.1 Preparation of the bone up to embedment

The bone preparations underwent the following processing steps up to being embedded in the block:

- 2 hours rinsing with flowing water
- 50 % ethanol 1 day
- 70 % ethanol 2-3 days
- 80 % ethanol 2-3 days
- 90 % ethanol 2-3 days
- 100 % ethanol 2-3 days
- 100 % ethanol 2-3 days
- acetone 2-3 days
- acetone 2-3 days
- acetone 2-3 days as required if not sufficiently degreased
- acetone -Methanol 2-3 days
- methanol 2-3 days

2.6.8.2 Embedding in methylmethacrylate

Following the methanol bath the preparations were transferred to the embedment in the following steps:

- Methylmethacrylate, pure, under vacuum for 2-3 days
- Methylmethacrylate, pure, under vacuum for 2-3 days
- Methylmethacrylate, pure, under vacuum for 2-3 days
- Methylmethacrylate mixture[4] under vacuum for 2-3 days
- Methylmethacrylate mixture in the incubator at approx. 25° C

2.6.8.3 Preparation of tissue sections

Preparation of tissue sections by microtome after decalcification. Embedment of the recovered bone preparations and staining with eosin followed by processing of the object slides for microscopy.

2.7 Note on the tests carried out

A comparison of the tests carried out with the clinical experiments described in the literature cited in this work shows no similarities, nor does it lead to any evidence-based results. The reasons for this include mainly the unclearly defined distance and time parameters in the publications up to now, as well as the fact that all these were carried out manually and ultimately that only xenogenous bone preparations were examined.

In the chapter Results manual testing and test assembly 2 are compared with each other. This comparison is permissible because the manual tests were carried out by the same person.

[4] Methylmethacrylate mixture: 900 ml methylmethacrylate (Merck 800590)
90 ml dibutyl phthalate (Merck 800919)
15 g Alpha-alpha-azo-iso-butyronitril (Merck 12431)

3. Results

3.1 Test assembly 1 compared with test assembly 2 in macroscopic representation

As all of the clinical experiments cited in the introduction [Lit. 1, 3] were carried out manually, a manual test was also carried out in order to make reference to clinical-macroscopic experiments.

This comparative manual test with the preparation series 1/0, 2/0, 3/0 and 4/0 was carried out with the chisel-shaped sapphire tip.

Work tip:	Tip C3 (see 2.5.1)
Handpiece:	Angle handpiece 90° (standard)
Power setting:	3.5 W (= 175 mJ/pulse), 20 Hz
H_2O/air setting:	Feed of water/air variable, see test protocol

After the experiment the preparations were immediately transferred to a fixing solution in a concentration of 8 % formaldehyde, 70 % undenatured alcohol.

3.1.1 Conditions for test assembly 1

All of the preparations were processed with Waterlase M. from the Biolase company.

Material	**Serial no.**	**Processing / Job**	**Power**	**Distance** tissue/ laser tip	**H_2O/air**	**Tip**
Maxillary preparation	1 / 0	manual	3.5 W = 175 mJ [5]	ca. 3 mm	55/65	C3
Maxillary preparation	2 / 0	manual	3.5 W = 175 mJ	ca. 3 mm	55/65	C3
Compacta regio mentalis	3 / 0	manual	3.5 W = 175 mJ	ca. 3 mm	55/65	C3
Compacta regio mentalis	4 / 0	manual	3.5 W = 175 mJ	ca. 3 mm	55/65	C3

Table 3: Conditions for test assembly 1

[5] Actual output at the solid body laser output as the condition of the handpiece mirror and the sapphire tip can reduce the output.

Conditions:

- Processing tip (chisel-shaped sapphire tip) not listed in annex.
- Fixing in 8 % formaldehyde / 70 % alcohol
- “Test conditions” as described in the literature, i.e. not calibrated.
- Not processed by machine, but manually.

  1st run of the tip at 60° ∠, subsequent still visible sections at 20° ∠ (approximate values).
- Tip distance to the preparation ca. 2 – 3 mm / room temperature 24° C.

3.1.2 Test material for test assembly 1

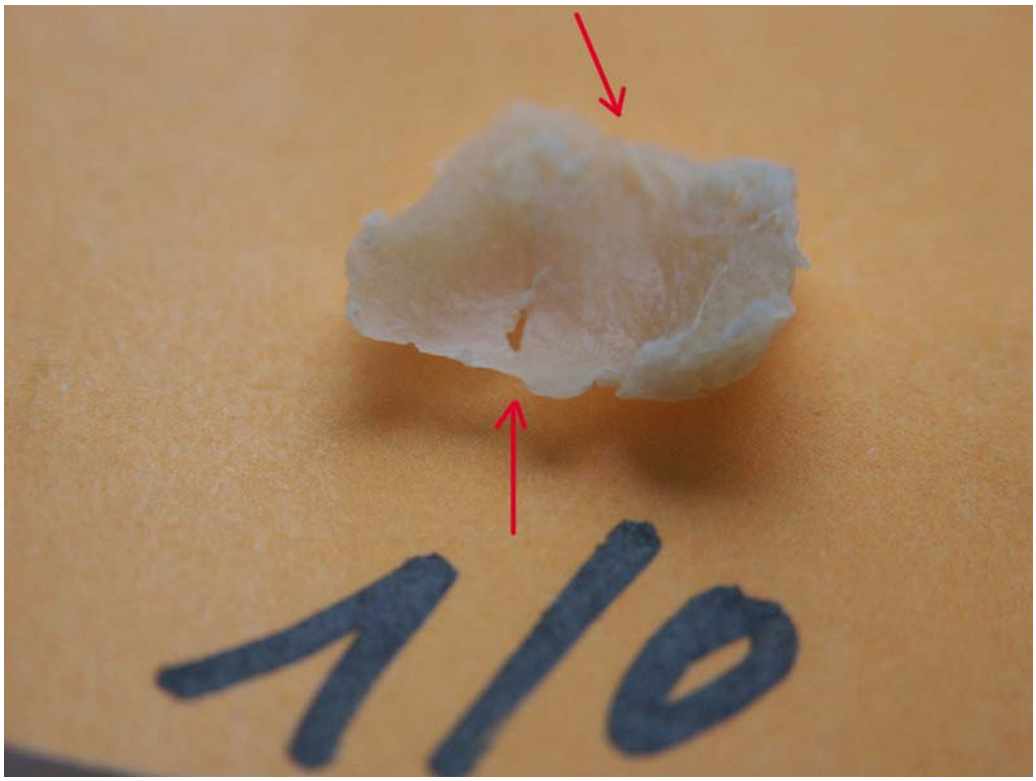


Fig. 9: Test material for manual test 1/0

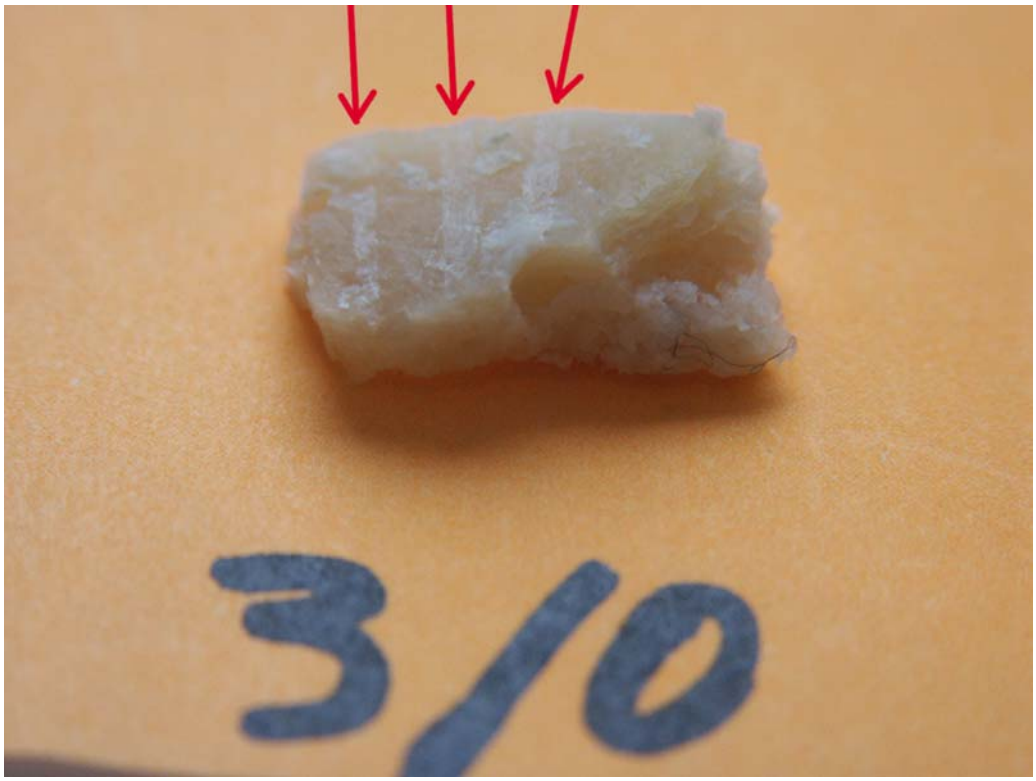


Fig. 10: Test material for manual test 3/0

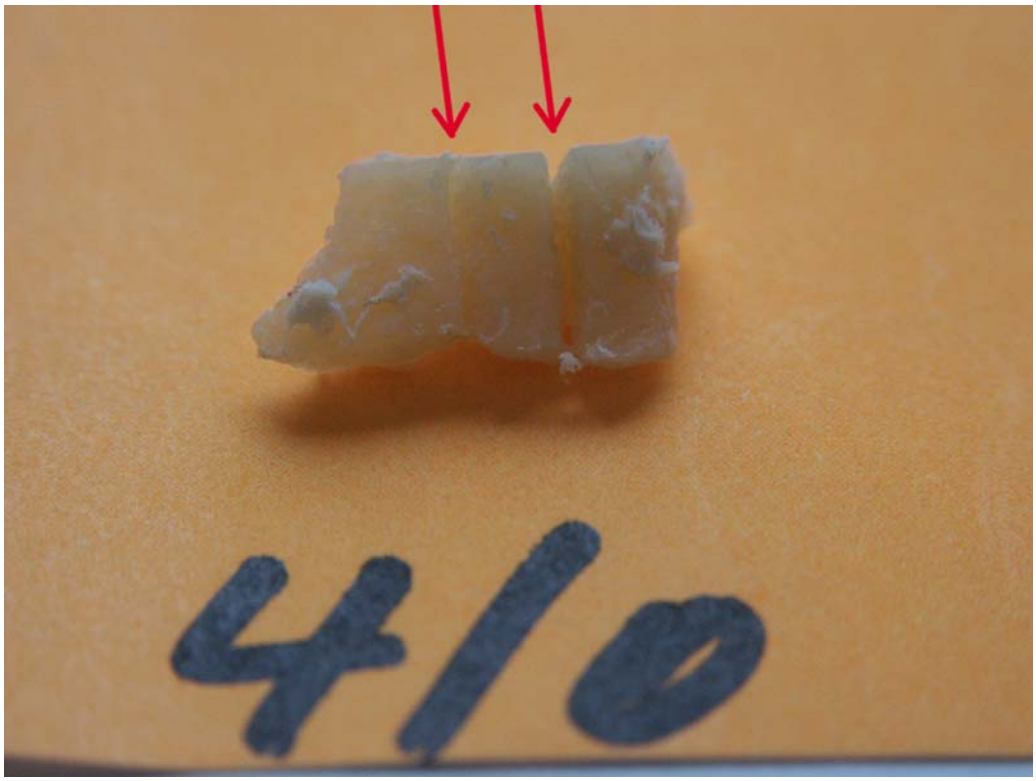


Fig. 11: Test material for manual test 4/0

3.1.3 Conditions for test assembly 2, preparation series A

All preparations processed with Waterlase M. from the Biolase company.

Material	Serial no.	Processing / Job	Power	Distance tissue/ laser tip	H_2O/air	Tip
Lower jaw left 6.11.04	A 1	24 mm_um4.job	5 W = 250 mJ [6]	3 mm	75/95	MG6
Lower jaw right 6.11.04	A 2	24 mm_um4.job	5 W = 250 mJ	3 mm	75/95	MG6
as then laser failure	A 2	but only 48,72,96	5 W = 250 mJ	3 mm	75/95	MG6
Maxillary right 9.11.04	A 3	24 mm_um3.job	3.5 W = 175 mJ [7]	3 mm	65/65	MG6
Maxillary left 9.11.04	A 4	24 mm_um2.job	3.5 W = 175 mJ	3 mm	65/65	MG6

Table 4: Preparations for test assembly 2, preparation series A

Conditions:

- Fixing in 8 % formaldehyde / 70 % alcohol
- Due to a defect in the laser the maxillary preparations were frozen (at –5° C) and only processed after thawing out on 9.11.04 (room temperature ca. 24° C).
- “Test conditions” not as described in the literature, but calibrated.
- Processed with the aid of test assembly 2, not manually.
  Set angle of the sapphire tip to the bone ca. 25°.
- Preparations A1 to A2 processed with distilled water at 62 ml/min, preparations A3 and A4 processed at 50 ml/min.

[6] Actual output at the solid body laser output as the condition of the handpiece mirror and the sapphire tip can reduce the output.

[7] Actual output at the solid body laser output as the condition of the handpiece mirror and the sapphire tip can reduce the output.

3.1.4 Test material for test assembly 2

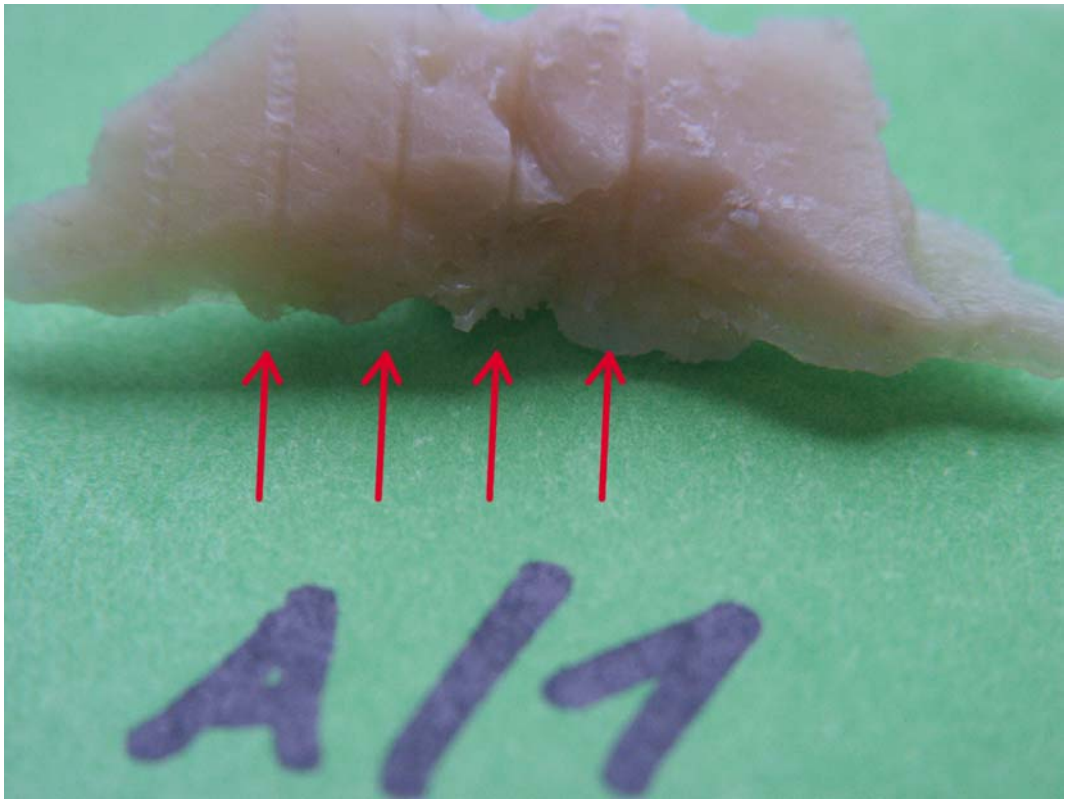


Fig. 12: Test material A/1

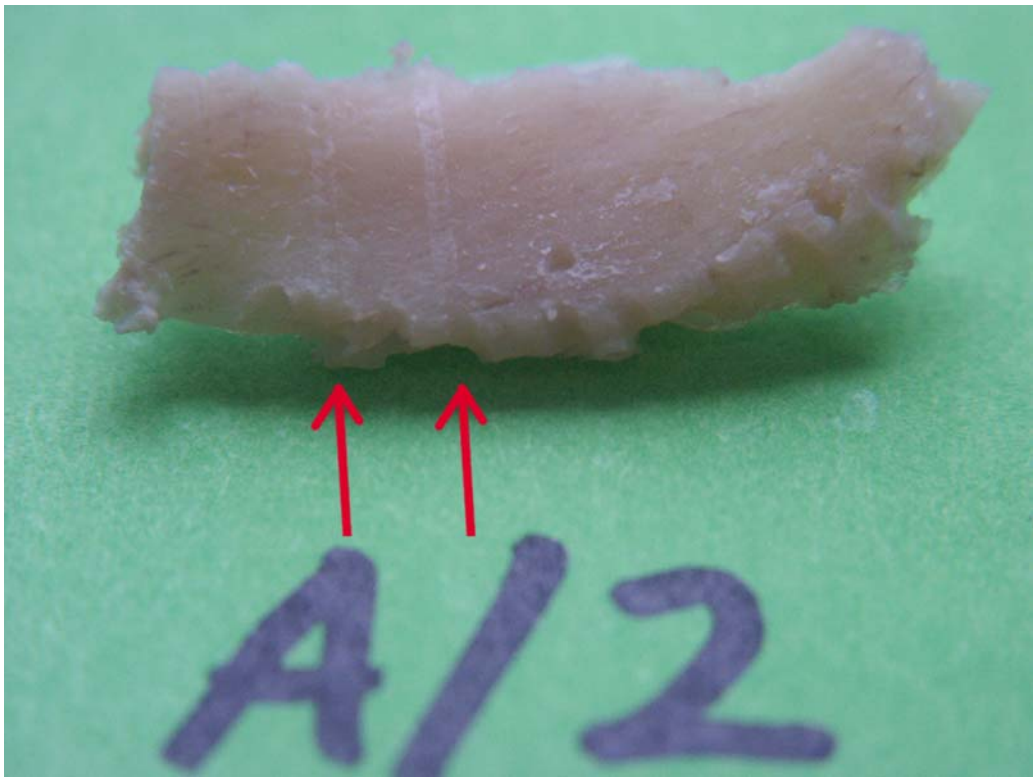


Fig. 13: Test material A/2

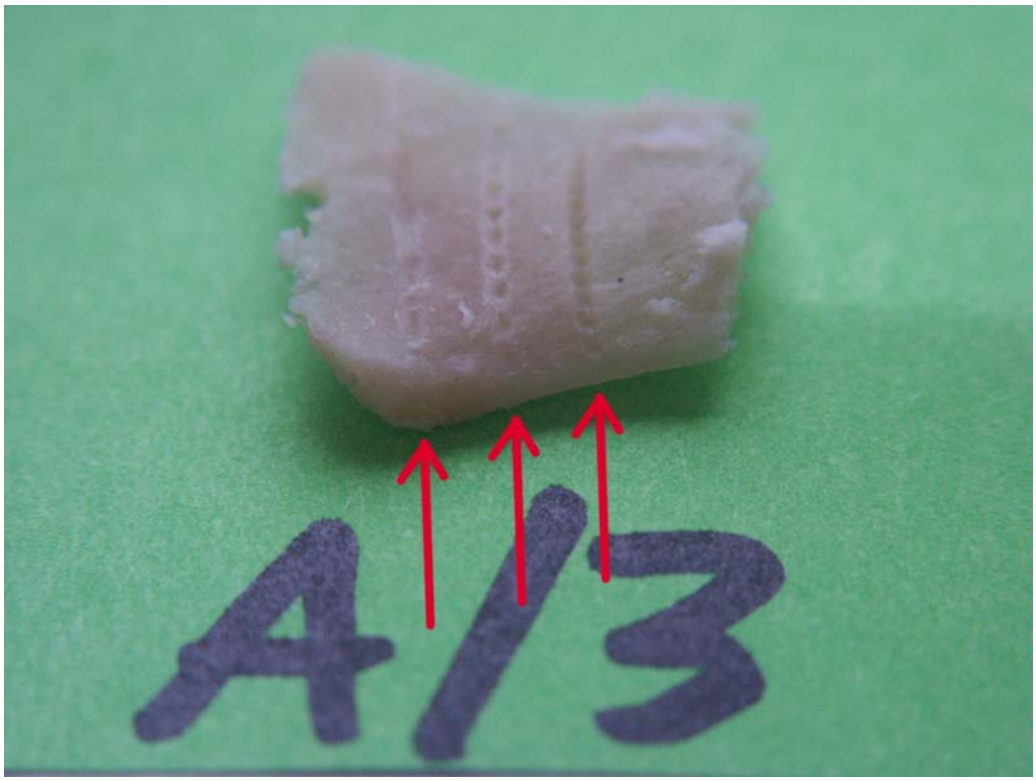


Fig. 14: Test material A/3

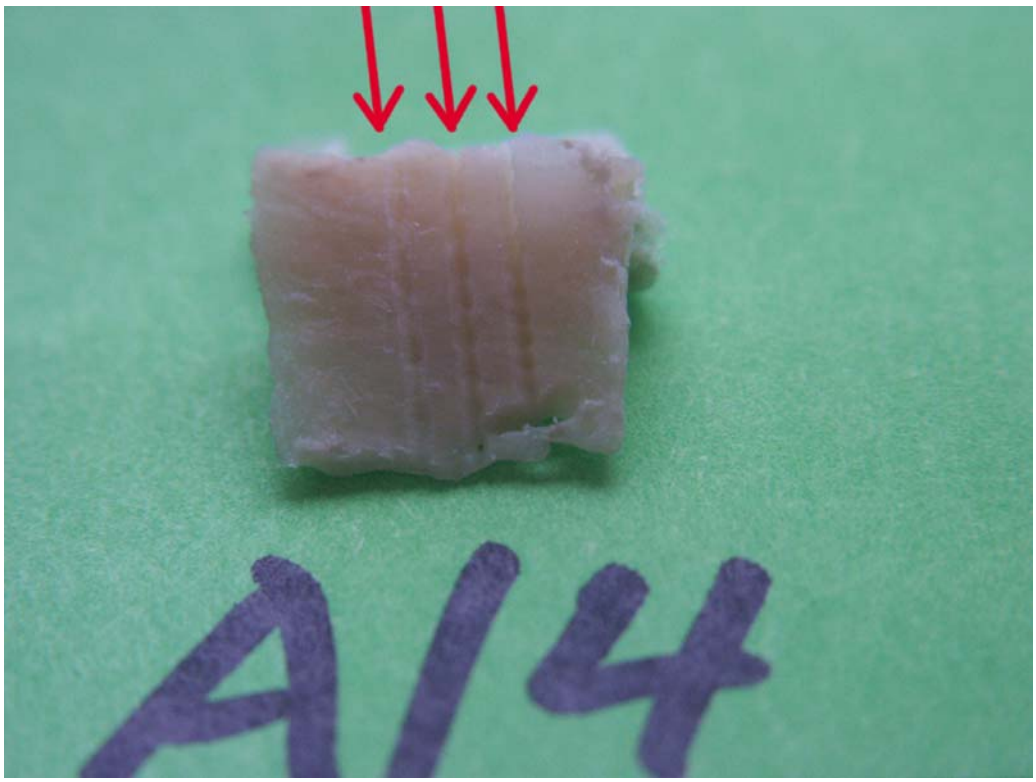


Fig. 15: Test material A/4

3.1.5 Conditions for test assembly 2, preparation series B

All preparations processed with Waterlase M. from the Biolase company.

Material	**Serial no.**	**Processing / Job**	**Power**	**Distance** tissue/ laser tip	**H_2O/air**	**Tip**
Compacta lower jaw right	B 1	24 mm_um4.job path run twice	3.5 W = 175 mJ [8]	3 mm	65/65	MG6
Maxillary preparation	B 2	24 mm_um3.job path run twice	3.5 W = 175 mJ	3 mm	65/65	MG6

Table 5: Conditions for test assembly 2, preparation series B

Conditions:

- Tests carried out at room temperature 24° C at 19:00 hrs → fixing with 4 % formaldehyde in 70 % alcohol.
- Room temperature ca. 24° C
- “Test conditions” not as described in the literature up to now, but calibrated.
- Processed with the aid of test assembly 2, not manually.
  Set angle of the sapphire tip to the bone ca. 25°.
- Preparations B1 and B2 processed with distilled water at 50 ml/min.

[8] Actual output at the solid body laser output as the condition of the fiber handpiece and the sapphire tip can reduce the output.

3.1.6 Test material for test assembly 2, series B

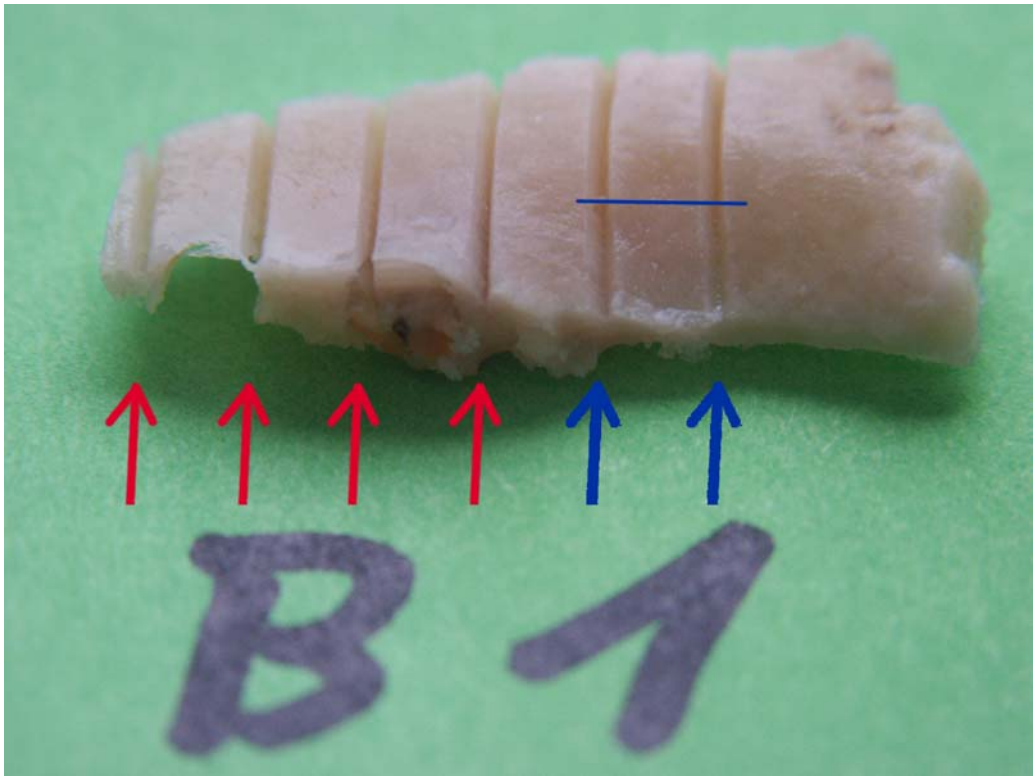


Fig. 16: Test material B1

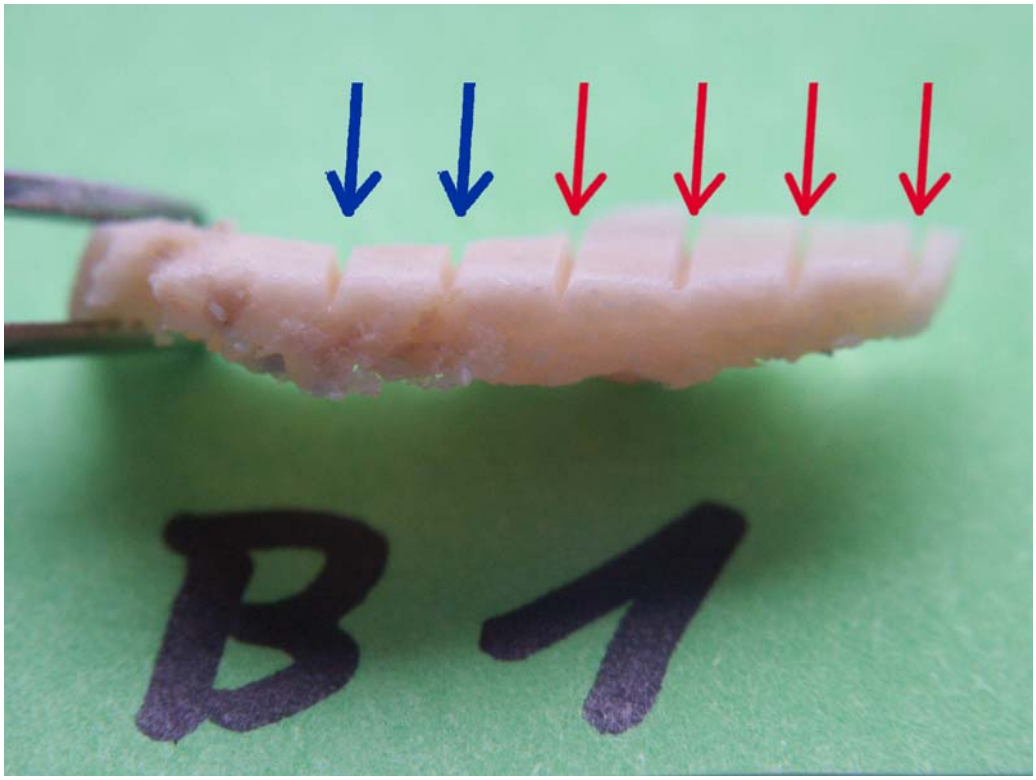


Fig. 17: Test material B1

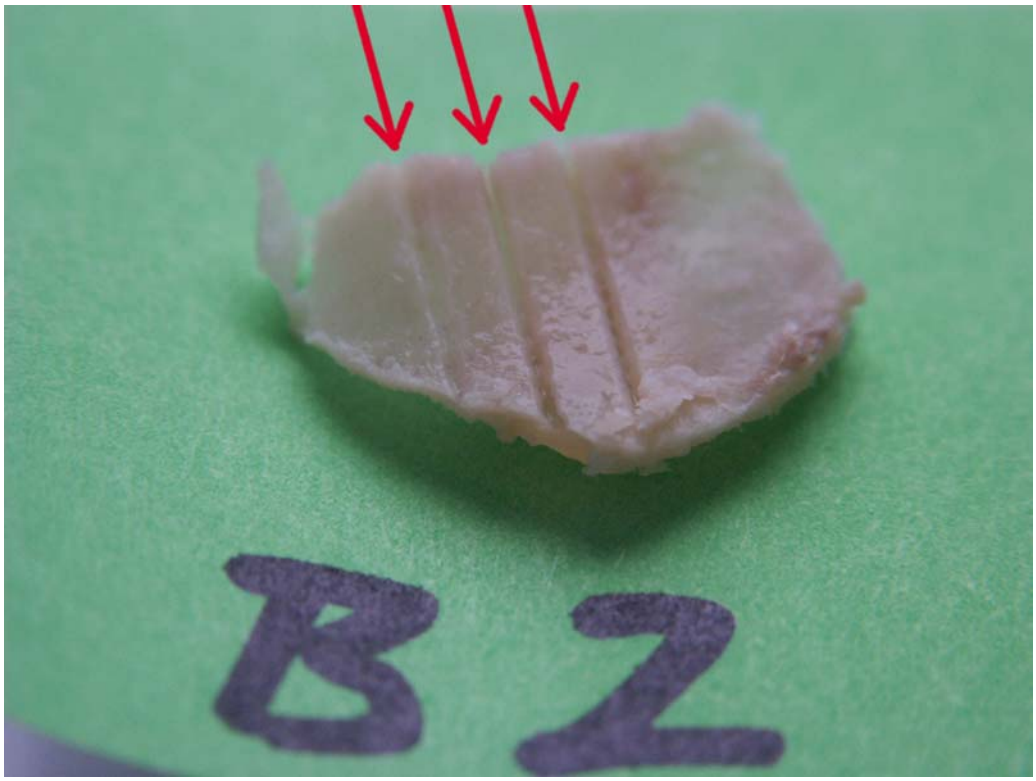


Fig. 18: Test material B2

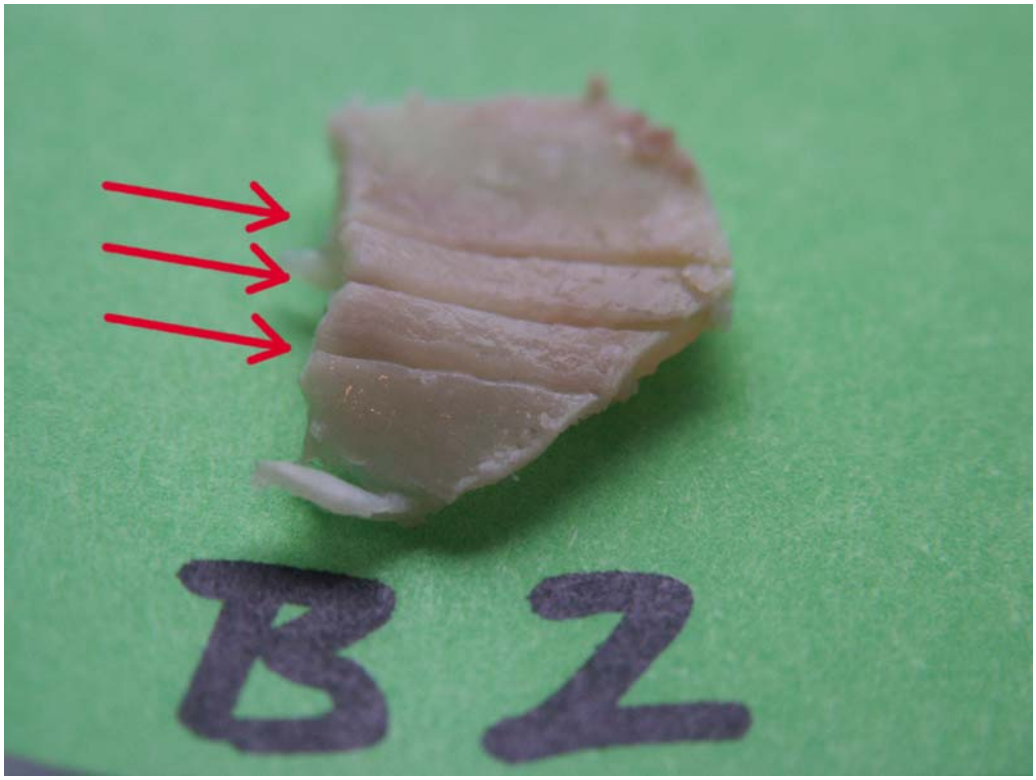


Fig. 19: Test material B2

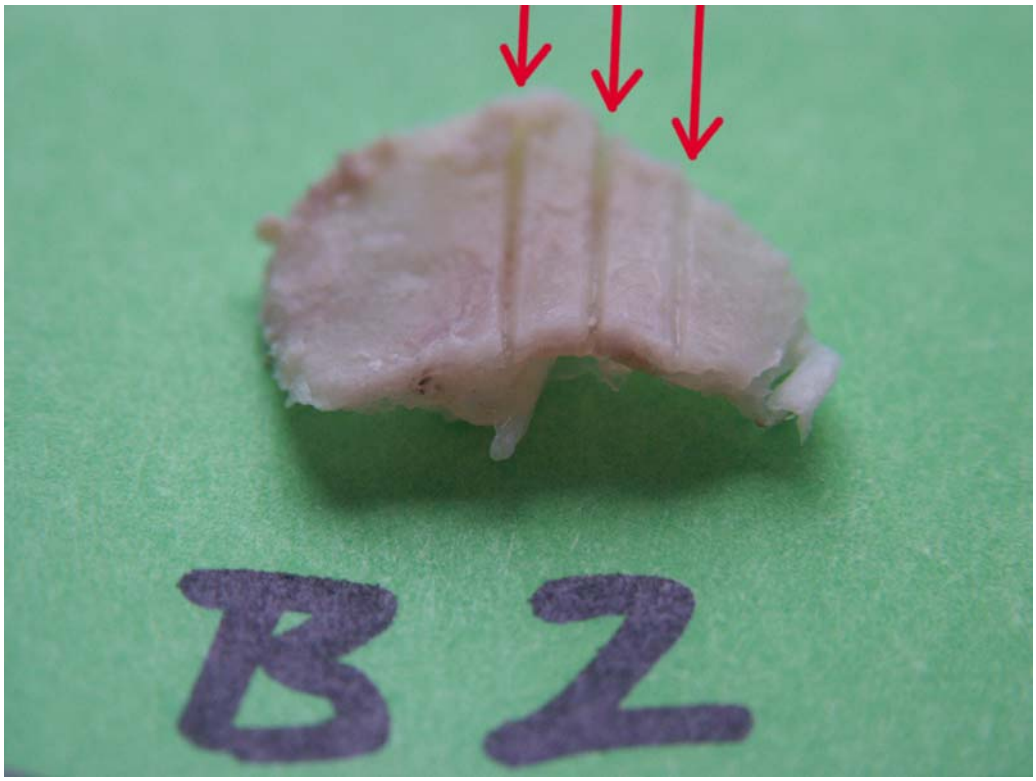


Fig. 20: Test material B2

3.2 Macroscopic evaluation of the test preparations

None of the bone preparations showed any signs of carbonization under 3.5 x magnification or the approx. 10 x screen enlargement (see test protocols in 3.1.1, 3.1.3, and 3.1.5).

In the comparison of laser preparation A1 and B1 it was established in B1 that the test assembly 2 executed its second run in the first two section levels. It was noticed that after the second run the double depth of the laser cut had not been reached.

The width of the section (3.2.1, Table 9) also changed insignificantly. This is because the laser effect had reached its maximum and, set out of focus, no clearly measurable removal of hard substance could be established.

Unfortunately there is no photographic documentation of preparation B1 for the result of the first run as this result of the experiment had not been expected.

3.2.1 Statistical evaluation

Statistical evaluation on the basis of photos to verify the achieved accuracy of the cut and the width of the laser exposure in the hard tissue.

Preparation	Distance[9] measured in the photo in mm			
	↔ 1	1 ↔ 2	2 ↔ 3	3 ↔ 4
A/1	1,57	1.60	1.,53	1.57
A/2		1.55		
A/3		1.14	1.08	
A/4		0.79	0.76	
B/1		1.56	1.57	1.57
B/2		1.10	1.23	

Table 6: Measured distances of the set values of 4, 3, and 2 mm.

Preparation	Expected distance	Distance[10] on the basis of actual width in mm			
		↔ 1	1 ↔ 2	2 ↔ 3	3 ↔ 4
A/1	4 mm	4.00	4.08	3.90	4.00
A/2	4 mm		4.00		
A/3	3 mm		3.08	2.92	
A/4	2 mm		2.04	1.96	
B/1	4 mm		3.98	4.01	4.01
B/2	3 mm		2.83	3.17	

Table 7: Expected distances of the set values of 4, 3 and 2 mm.

Preparation	Measured width[11] of the laser exposure in mm				
	0	1	2	3	4
A/1	0.35	0.30	0.28	0.14	0.17
A/2		0.27	0.25		
A/3		0.28	0.31	0.23	
A/4		0.23	0.25	0.20	
B/1		0.20	0.21	0.22	0.25
B/2		0.27	0.28	0.29	

Table 8: Measured width of the laser exposure.

[9] Measured with the help of auxiliary lines at strong magnification always at the centre of the preparation (best resolution) and in the center of the laser incision.

[10] On the basis of the mean value and the width set in the control program (see 3.1.3, 3.1.5 and 3.1.6).

[11] Measured with the help of auxiliary lines at strong magnification always at the centre of the preparation (best resolution) and in the center of the laser incision.

Note: The values 0.14 and 0.17 in preparation A/1 were not included in the evaluation because the working plane of the laser was not correctly focused on the tissue.

Preparation	Expected distance	Calculated width[12] of the laser exposure in mm						
		0	1	2	3	4	5	6
A/1	4 mm	0.89	0.77	0.71				
A/2	4 mm		0.70	0.65				
A/3	3 mm		0.76	0.84	0.62			
A/4	2 mm		0.59	0.65	0.52			
B/1 (plan view)	4 mm		0.79	0.79	0.68	0.68	0.79	0.79
B/2	3 mm		0.70	0.72	0.75			

Table 9: Calculated width of the laser exposure.

The mean laser cut width with a G6 tip thus amounts to 0.72 mm, calculated from 20 values of the preparations A/1 to B/2, independent of whether the power was set at 5 Watt or 3.5 Watt.

This mean value also served for the representation of the scale in Fig. 50 and Fig. 51.

3.3 Registration of the absolute performance in mJ/mm/sec = mW/mm

In the older literature the outputs and the set treatment values of the lasers are always stated in Watt, today almost exclusively in mJ/pps [pulses per second]. These stated output parameters however, only actually describe the nominal output of the laser, but not the actual output at the working tip. Some lasers – including the dental lasers, the Er,Cr:YSGG laser and the Er:YAG laser – are subject to losses if the cross section of the focus formed in the handpiece is larger than the diameter of the laser tip used.

For this reason the Biolase company gives a calibration factor of 1.0 or less for each working tip and thus states the actual output at the working tip.
If, for example, the calibration factor of a laser is 0.5 and the set output 6 Watt, 20 Hz, then the actual output at the working tip amounts to 3 Watt, 20 Hz.

[12] Width set on the basis of the mean value and in the control program (see 3.1.3, 3.1.5 and 3.1.6).

For this reason it is advisable to measure the actual output at the working tip in experiments as the values can fluctuate even in devices of the same model. The type of handpiece and the working tip also influence the output.

Publications up to now, even in the period from 2004 to 2007, do not describe the output per path run. The parameters path and time during which the energy is applied to the tissue are thus not registered. Occasionally stated energy densities per square centimeter are of no use, if distance and path have not been registered in terms of time units. The following table puts the effect of the energy in relation to path and time (see column 'mW/mm'). As we know that the working track processed by the tip MG6 is approximately 0.7 mm wide and the path taken is 1 mm, the actual quantity of the energy on the tissue is thus mW/0.7 mm² (see below).

Preparation	Path	mm	sec	mJ	Hz	mW/mm	mW/0.7 mm²
A/1	1	1	2	250	20	10,000	10,000
	2	1	3	250	20	15,000	15,000
	3	1	4	250	20	20,000	20,000
	4	1	5	250	20	25,000	25,000
A/2[13]	1	1	2	250	20	10,000	10,000
	2	1	3	250	20	15,000	15,000
A/3	1	1	2	175	20	7,000	7,000
	2	1	3	175	20	10,500	10,500
	3	1	4	175	20	14,000	14,000

[13] Only two paths due to laser defect; output reduction must be assumed.

Preparation	Path	mm	sec	mJ	Hz	mW/mm	mW/0.7 mm²
A/4	1	1	1	175	20	3,500	3,500
	2	1	2	175	20	7,000	7,000
	3	1	3	175	20	10,500	10,500
B/1	1	1	2 x 2	175	20	2 x 7,000	2 x 7,000
	2	1	2 x 3	175	20	2 x 10,500	2 x 10,500
	3	1	2 x 4	175	20	2 x 14,000	2 x 14,000
	4	1	2 x 5	175	20	2 x 17,500	2 x 17,500
B/2	1	1	1	175	20	3,500	3,500
	2	1	2	175	20	7,000	7,000
	3	1	3	175	20	10,500	10,500

Table 10: Registration of the laser output in mW/mm

3.4 Microscopic evaluations of the test preparations

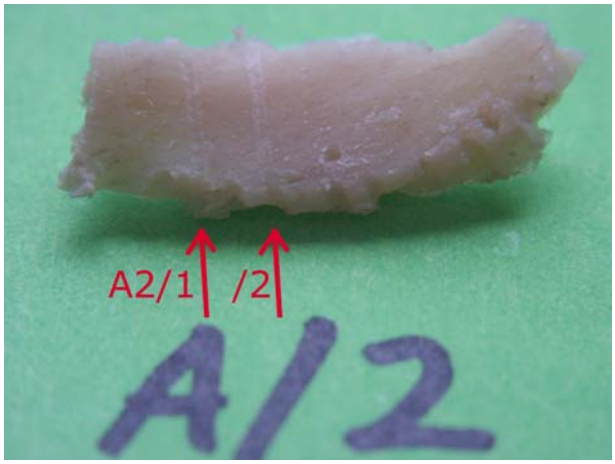
The red arrows point to the paths run by the laser over the bone.

The ablation depth of the Er,Cr:YSGG in the hard tissue amounts to ca. 100 µm at a penetration depth of ca. 3 µm in H_2O. Among other things, it is clearly dependent on the focus (distance of working tip from tissue) and from the proportion of water in the spray.

3.4.1 Preparations in histological preparation and view

The manually processed preparations 1/0, 3/0 and 4/0 were not included in the histological processing for the reasons stated (see 2.2.2) .

3.4.1.1 Preparation A/2



The red arrows point to the paths processed.

Working planes 1 to 2:

Path 1 run: 1 mm in 2 sec.

Path 2 run: 1 mm in 3 sec.

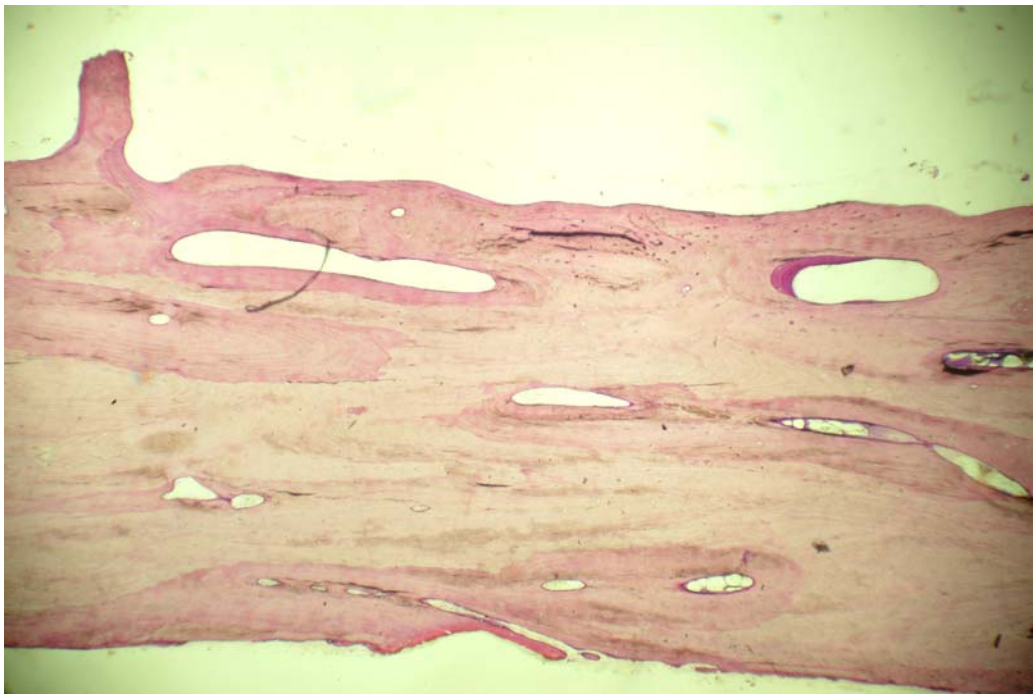


Fig. 21: A2/1 – Magnification factor 4 x

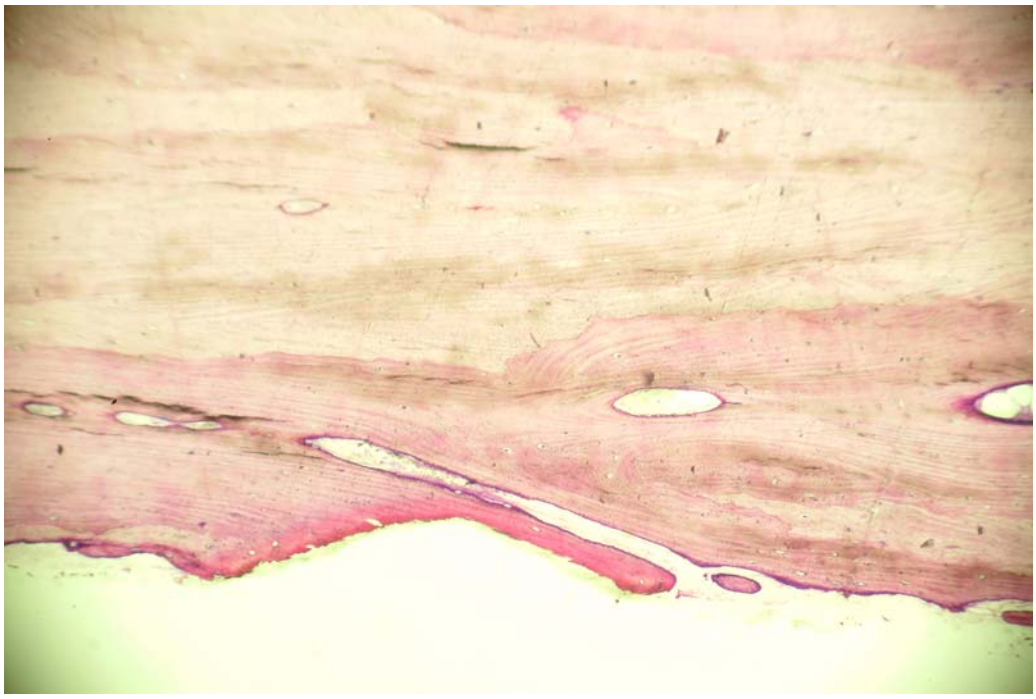


Fig. 22: A2/1 – Magnification factor 10 x

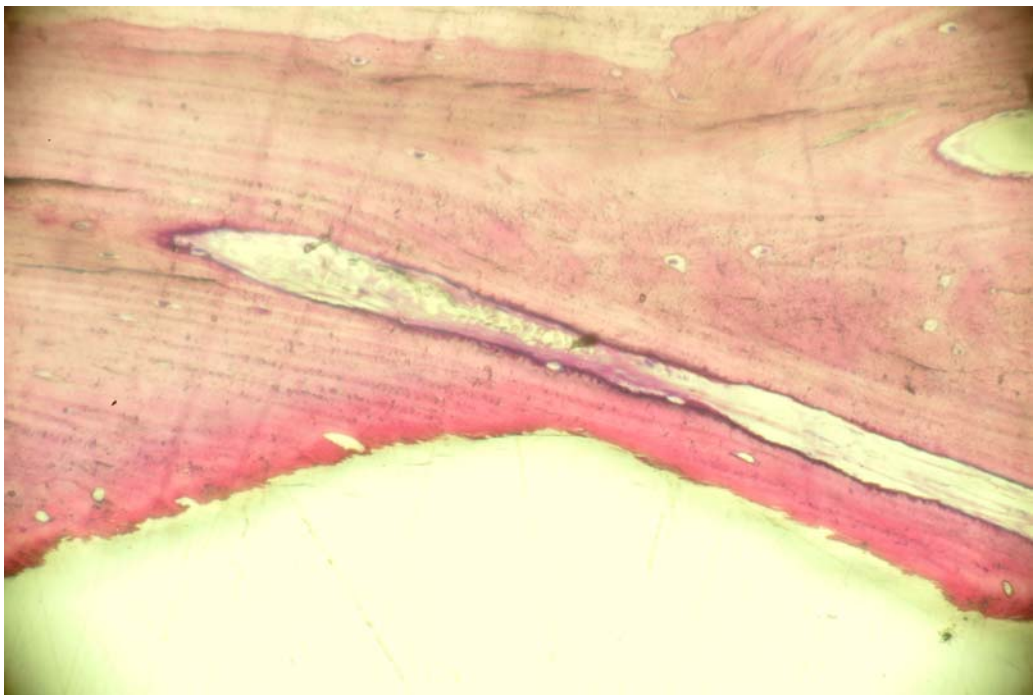


Fig. 23: A2/1 – Magnification factor 25 x

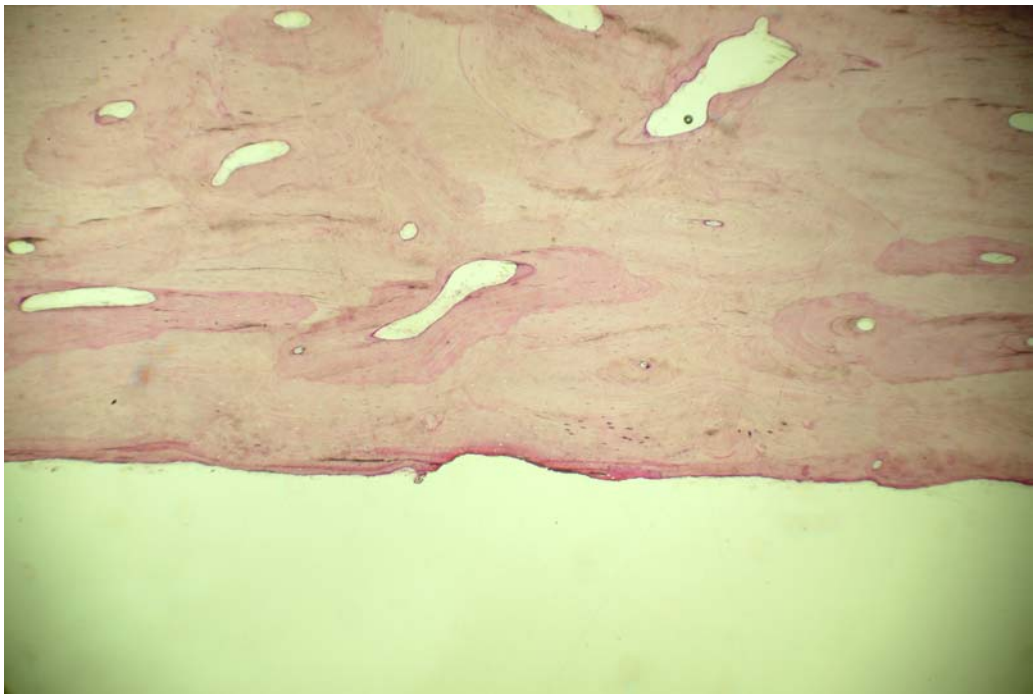


Fig. 24: A2/2 – Magnification factor 4 x

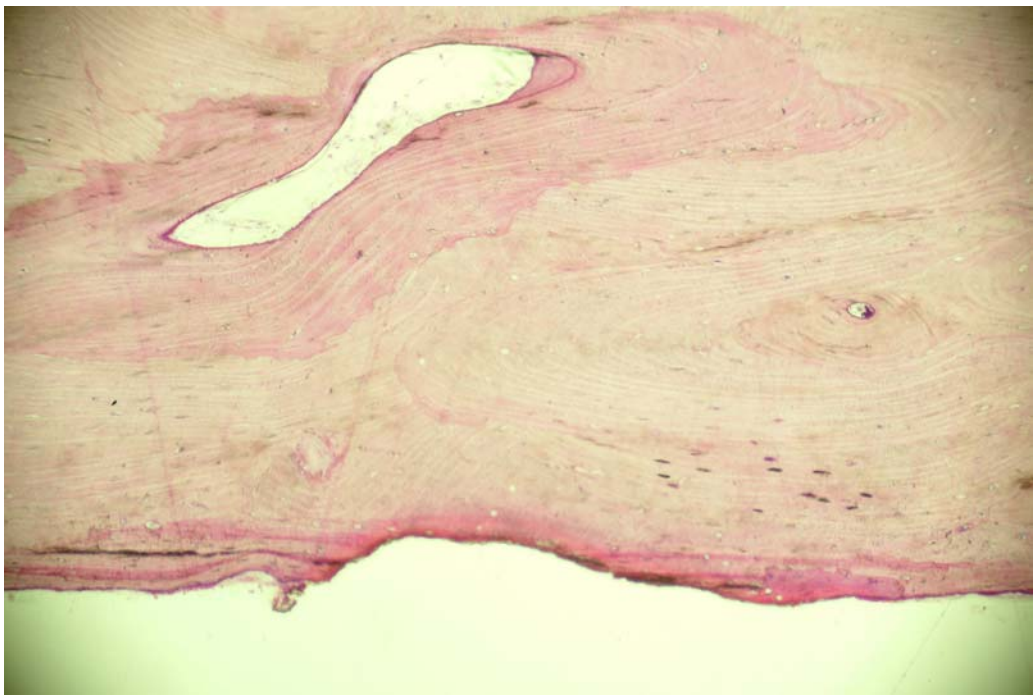


Fig. 25: A2/2 – Magnification factor 10 x

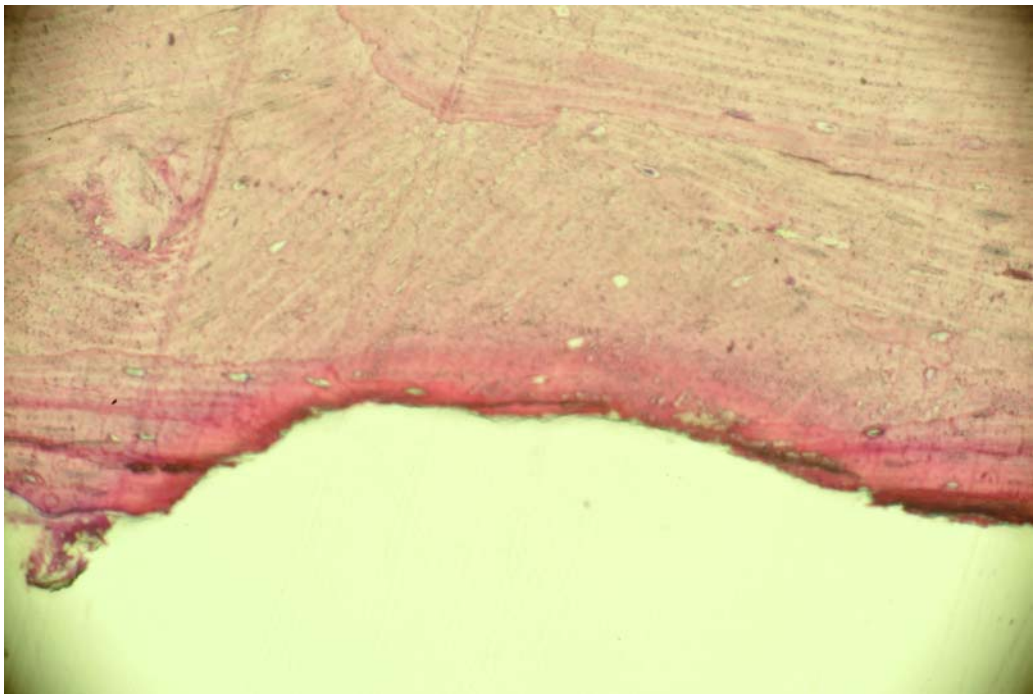


Fig. 26: A2/2 – Magnification factor 25 x

3.4.1.2 Preparation A/3

Working planes 1 to 3:

Path 1 run: 1 mm in 2 sec.

Path 2 run: 1 mm in 3 sec.

Path 3 run: 1 mm in 4 sec.

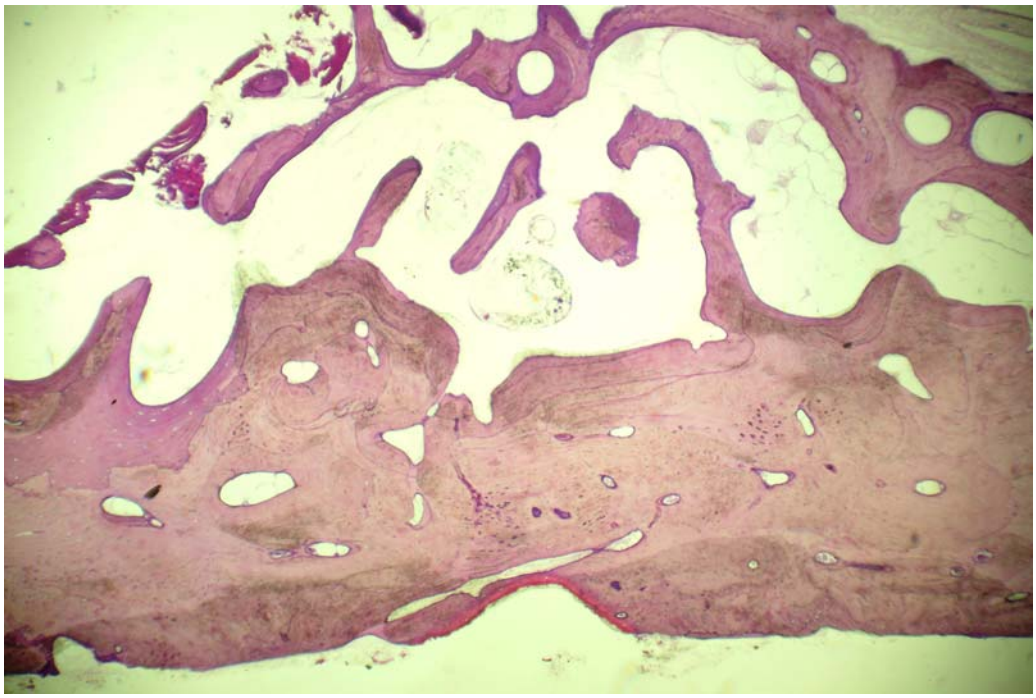


Fig. 27: A3/1 – Magnification factor 4 x

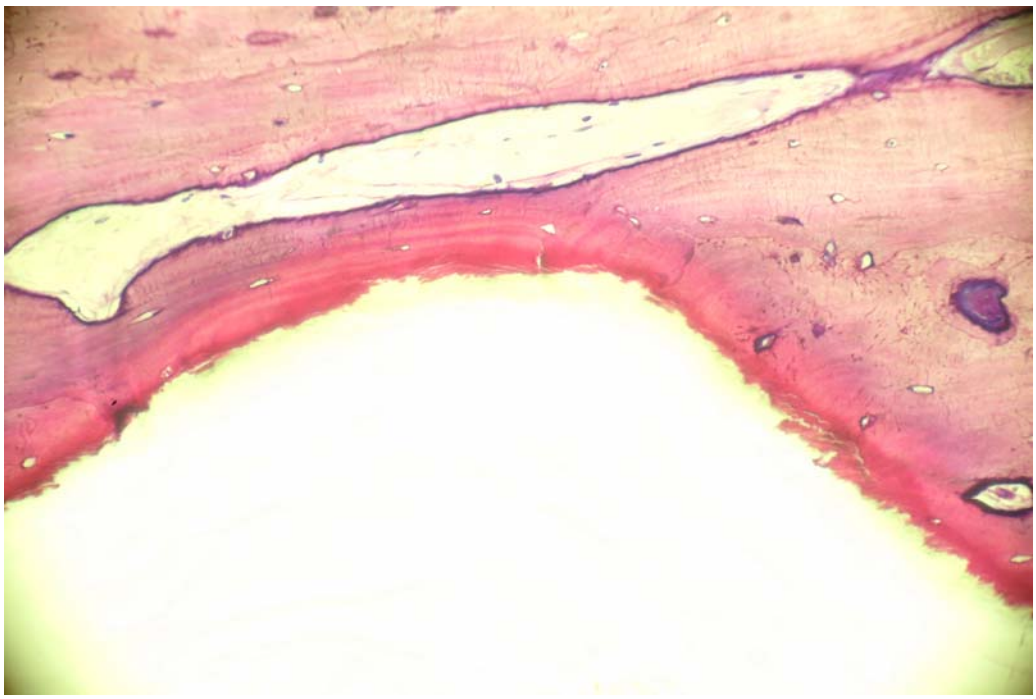


Fig. 28: A3/1 – Magnification factor 25 x

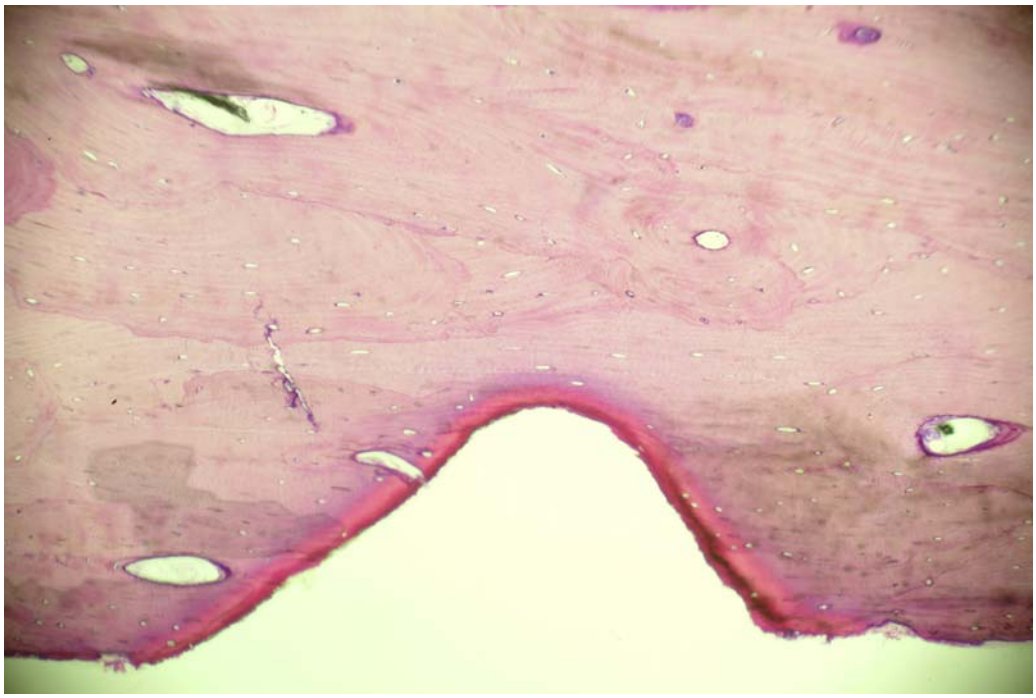


Fig. 29: A3/2 – Magnification factor 10 x

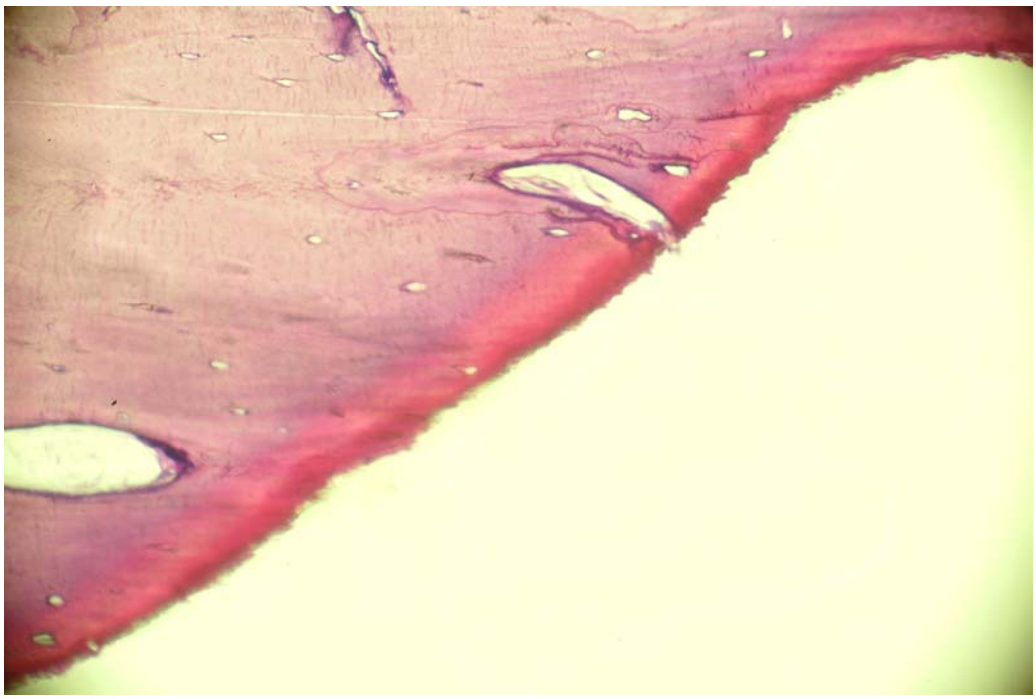
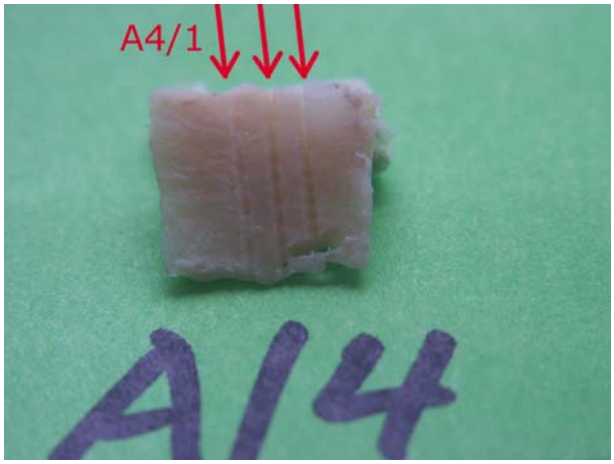


Fig. 30: A3/2 – Magnification factor 25 x

3.4.1.3 Preparation A/4



Working planes 1 to 3:

Path 1 run: 1 mm in 1 sec.

Path 2 run: 1 mm in 2 sec.

Path 3 run: 1 mm in 3 sec.

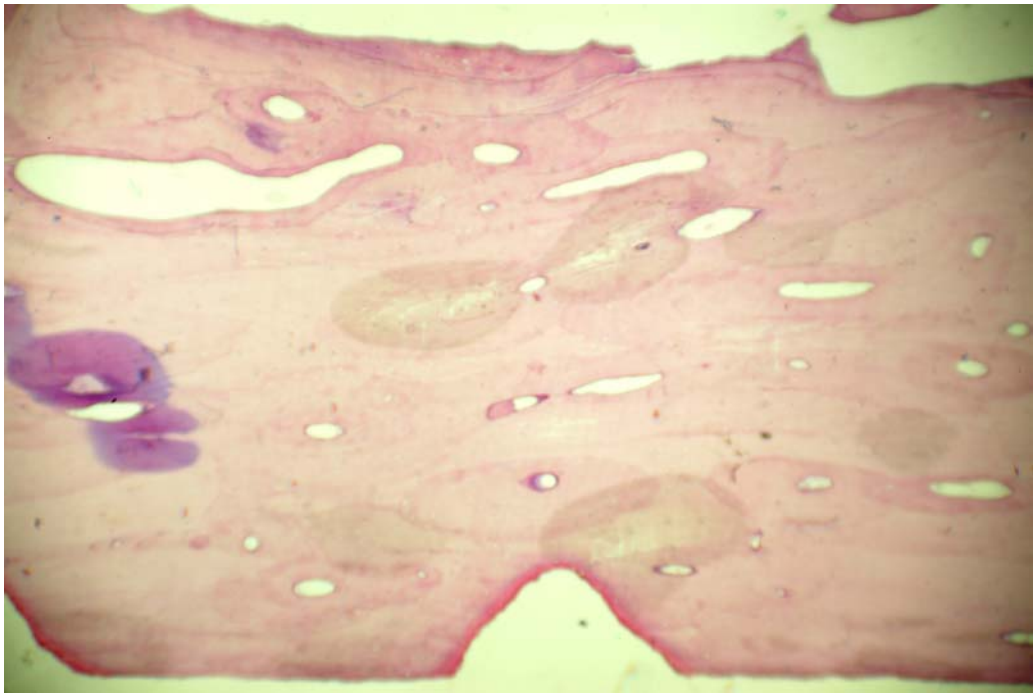


Fig. 31: A4/1 – Magnification factor 4 x

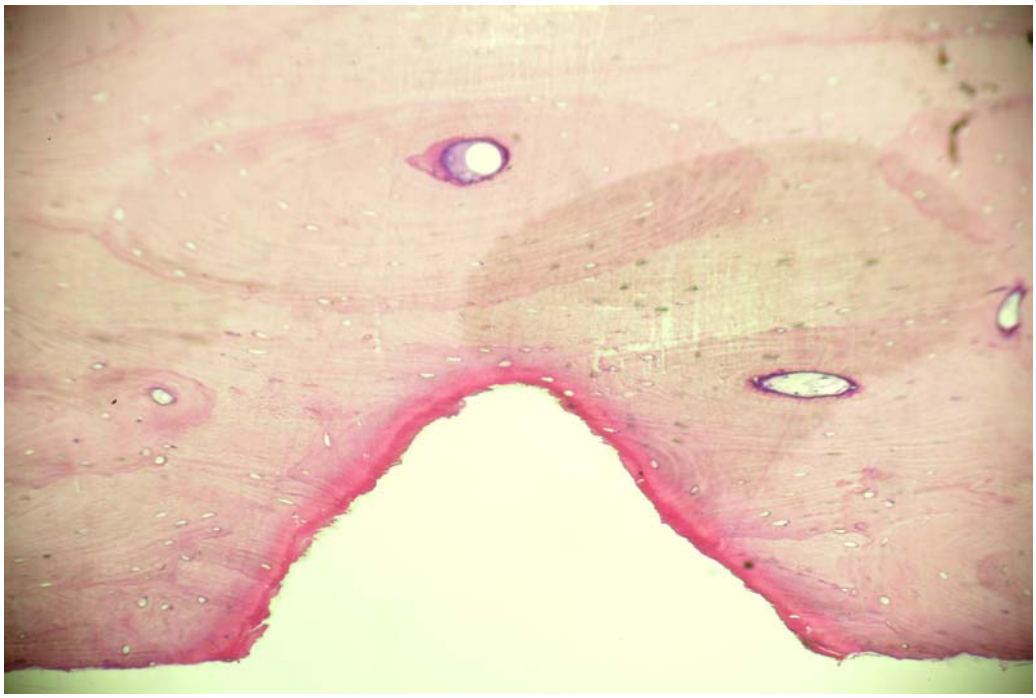


Fig. 32: A4/1 – Magnification factor 10 x

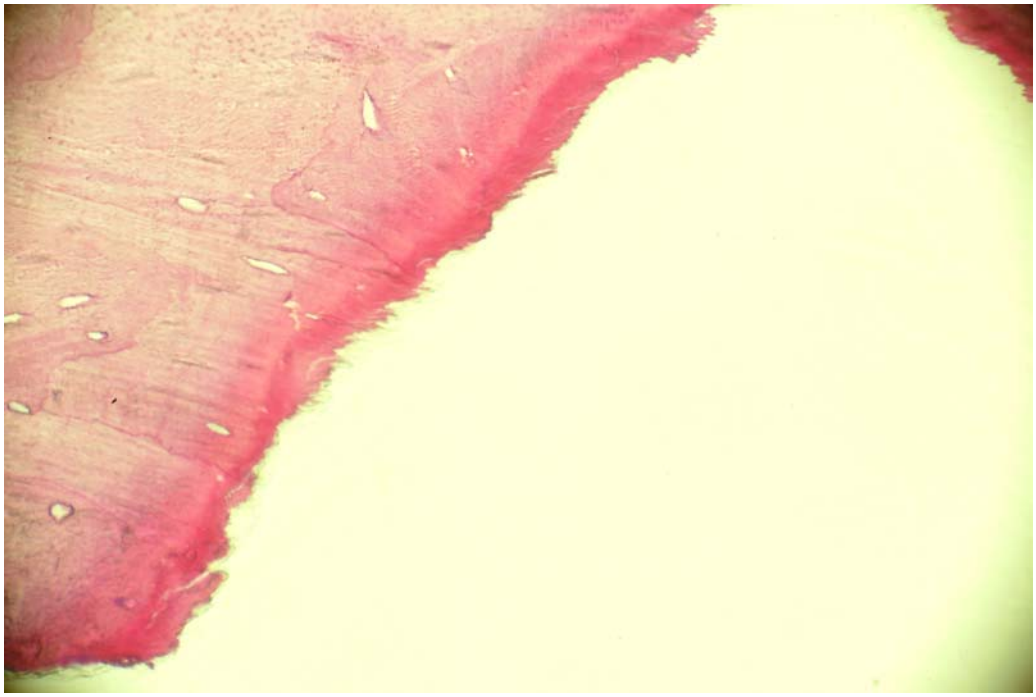


Fig. 33: A4/1 – Magnification factor 25 x

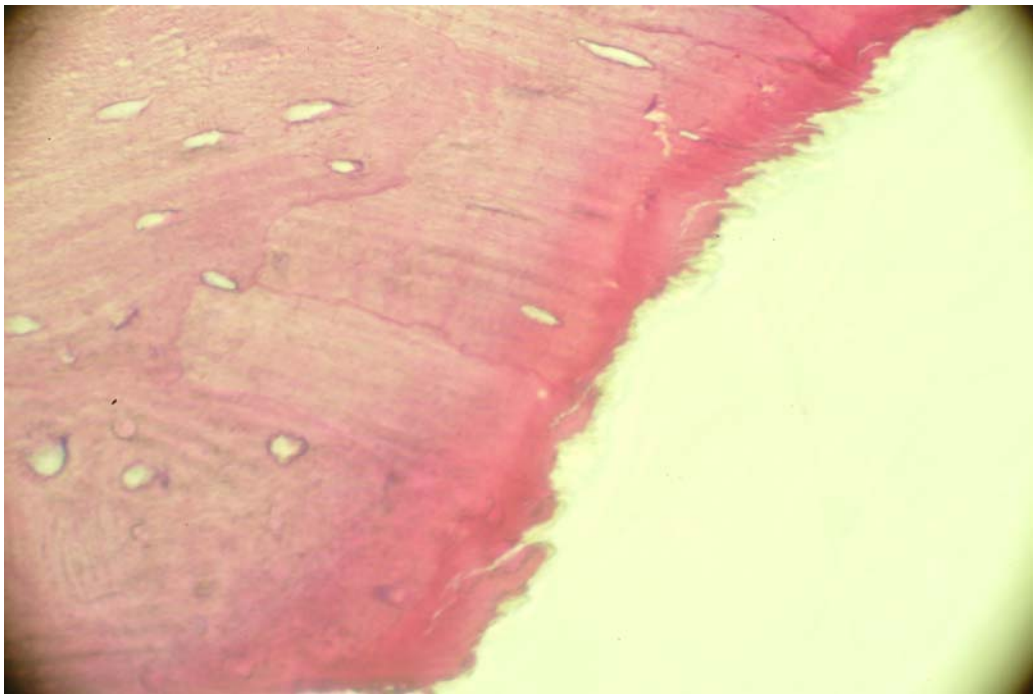


Fig. 34: A4/1 – Magnification factor 40 x

3.4.1.4 Preparation A/1

Working planes 1 to 4: Path 1 run: 1 mm in 2 sec.
Path 2 run: 1 mm in 3 sec.
Path 3 run: 1 mm in 4 sec.
Path 4 run: 1 mm in 5 sec.

Distance of the laser work point (600 µm Sapphire): 3 to 3.5 mm

Laser Settings: 5 Watt = 250 mJ
distilled water 75 %, compressed air 95 %
angle of the laser point to the preparation 25°

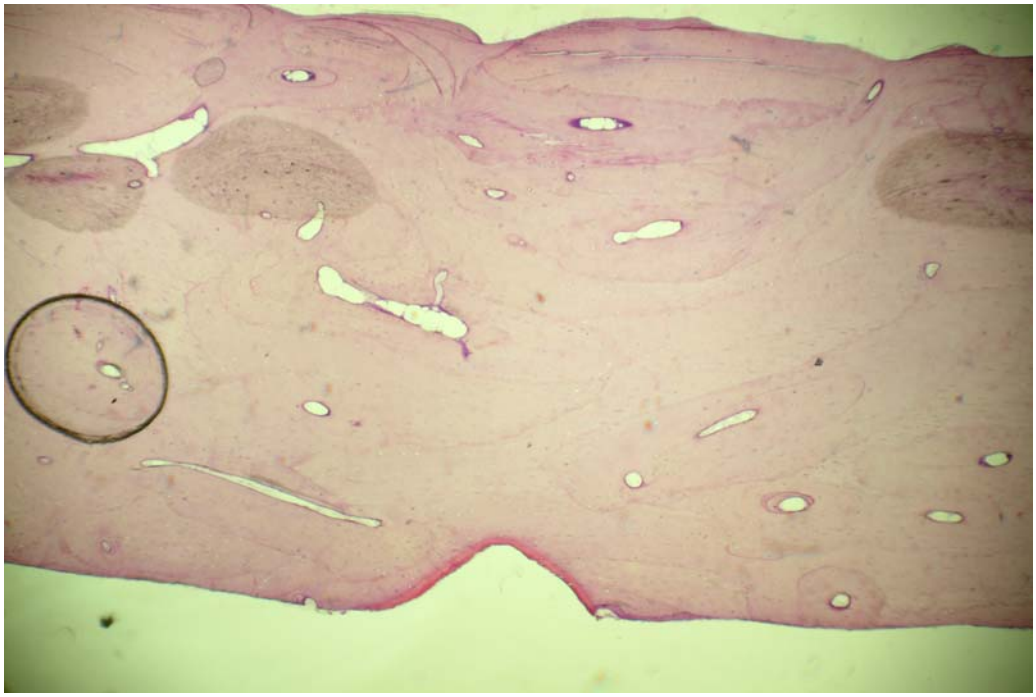


Fig. 35: A1/1 – Magnification factor 4 x

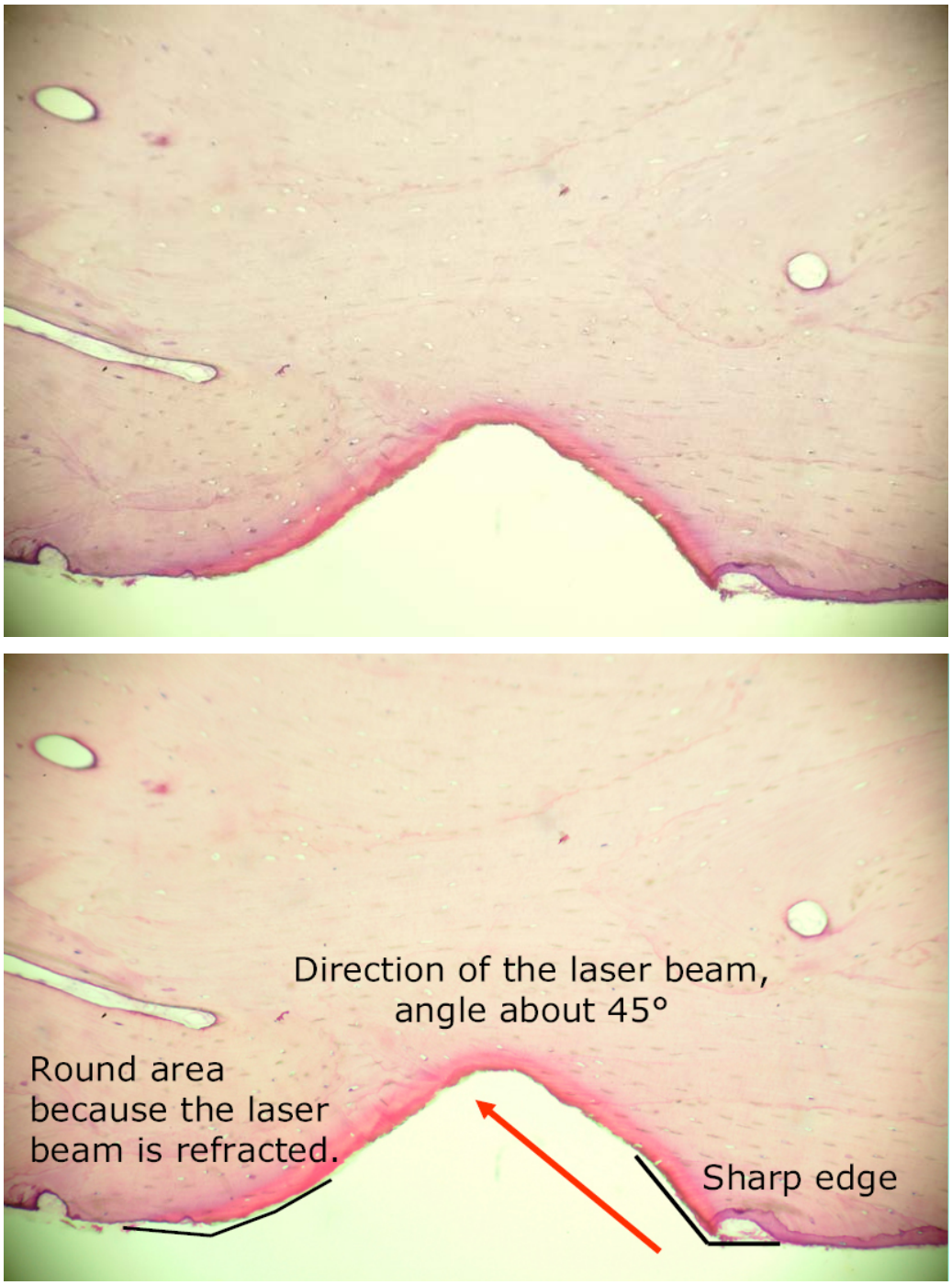


Fig. 36 a and b: A1/1 – Magnification factor 10 x (and explanation)

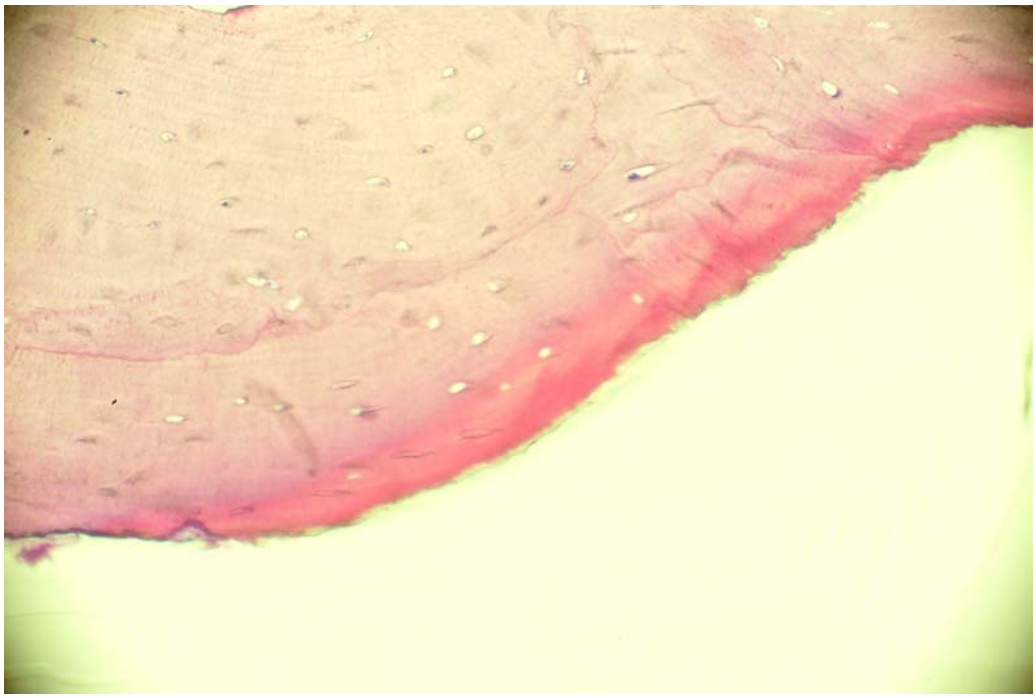


Fig. 37: A1/1 – Magnification factor 25 x

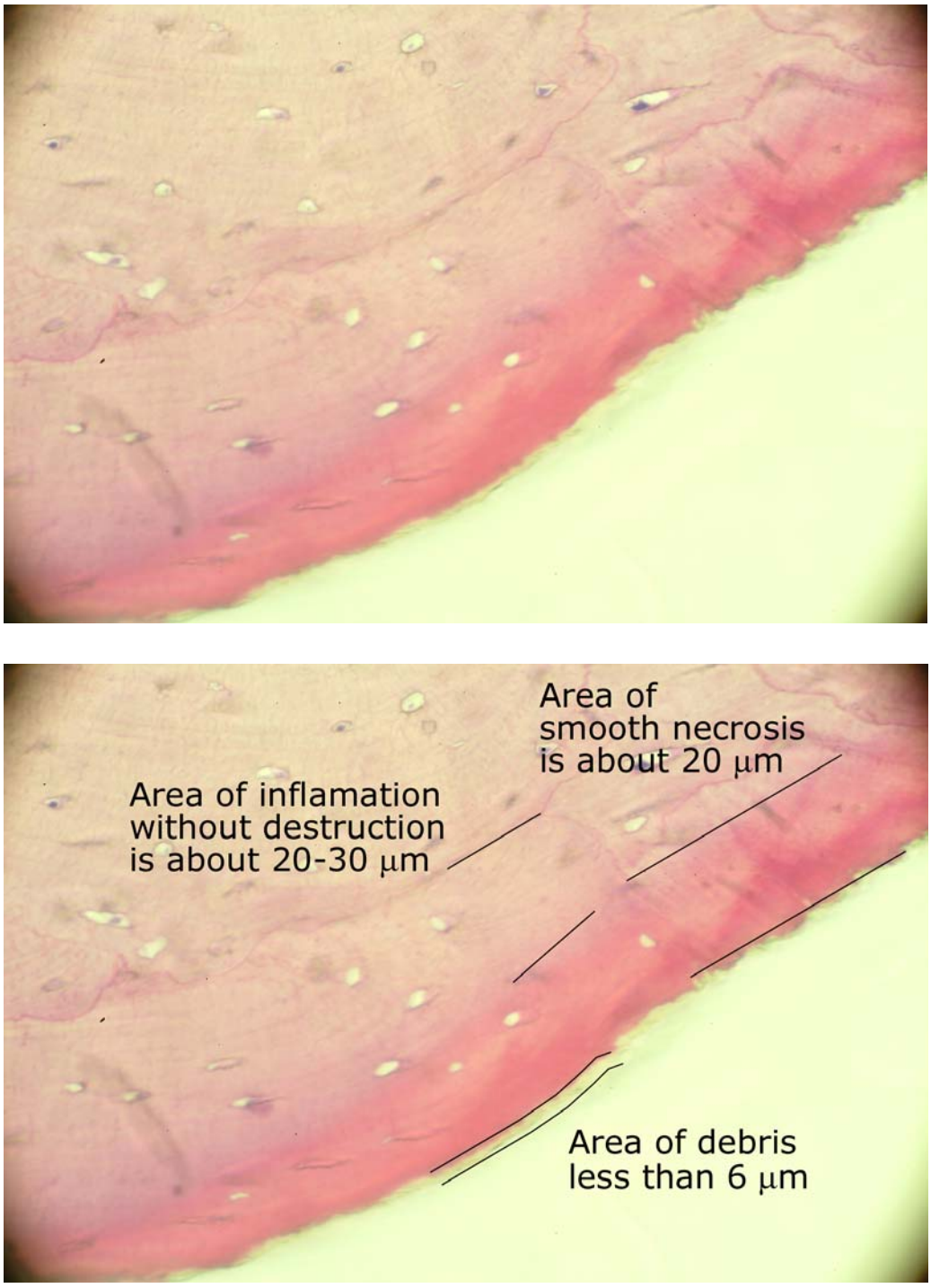


Fig. 38 a and b: A1/1 – Magnification factor 40 x (and explanation)

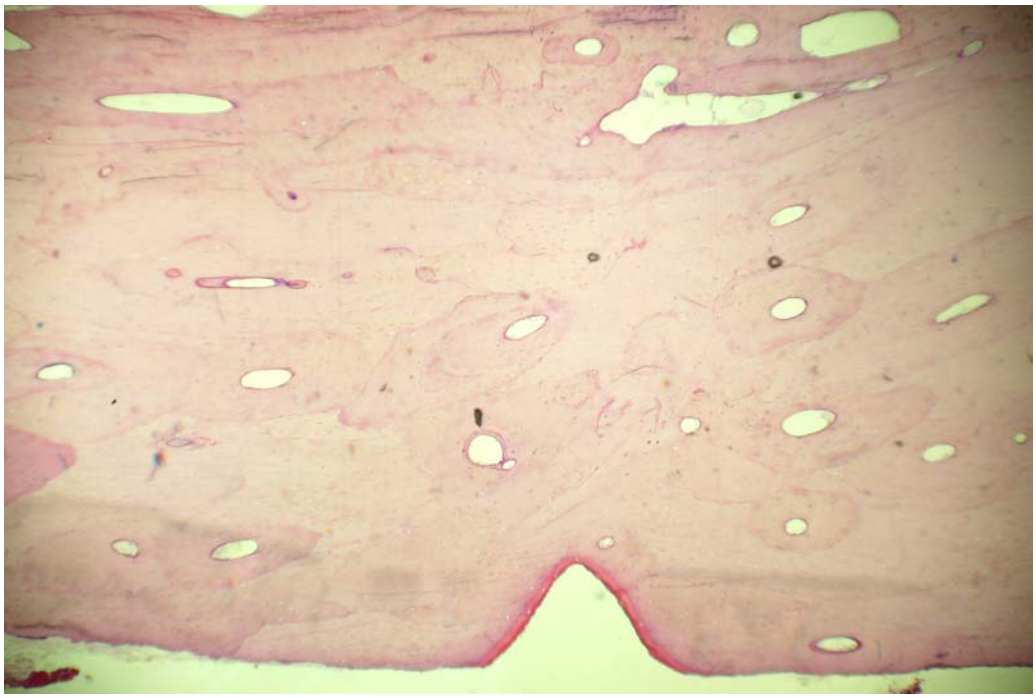


Fig. 39: A1/2 – Magnification factor 4 x

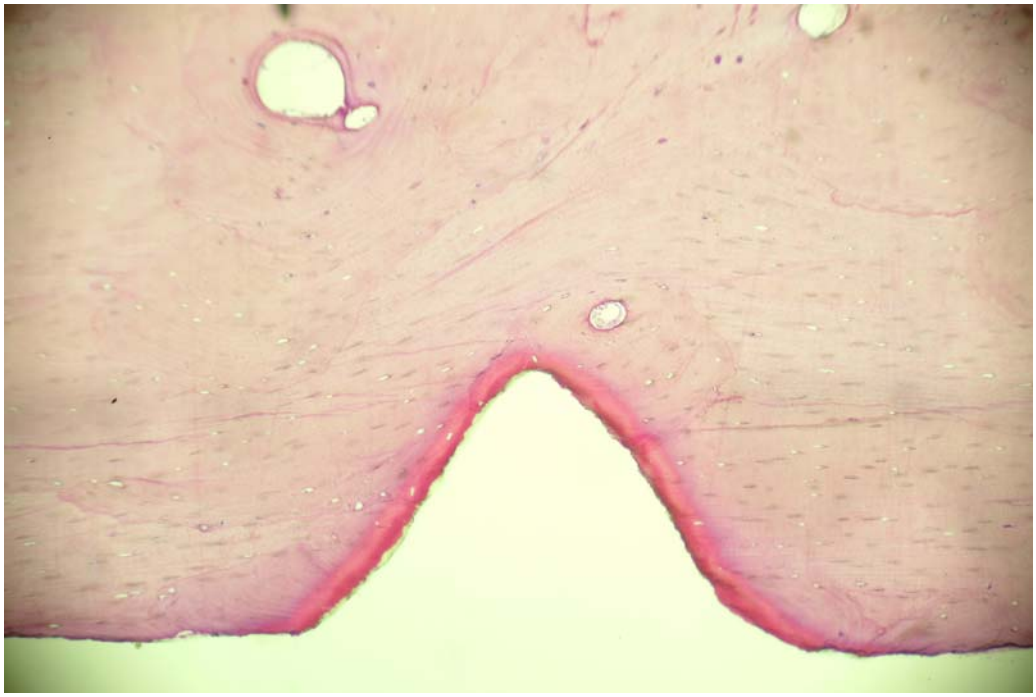


Fig. 40: A1/2 – Magnification factor 10 x

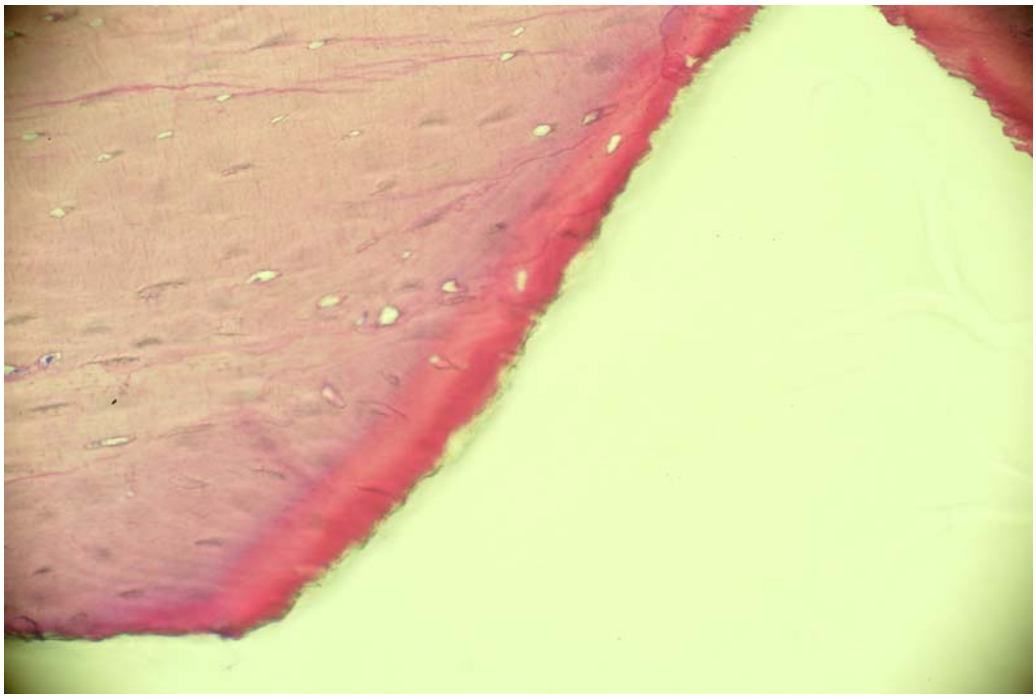


Fig. 41: A1/2 – Magnification factor 25 x

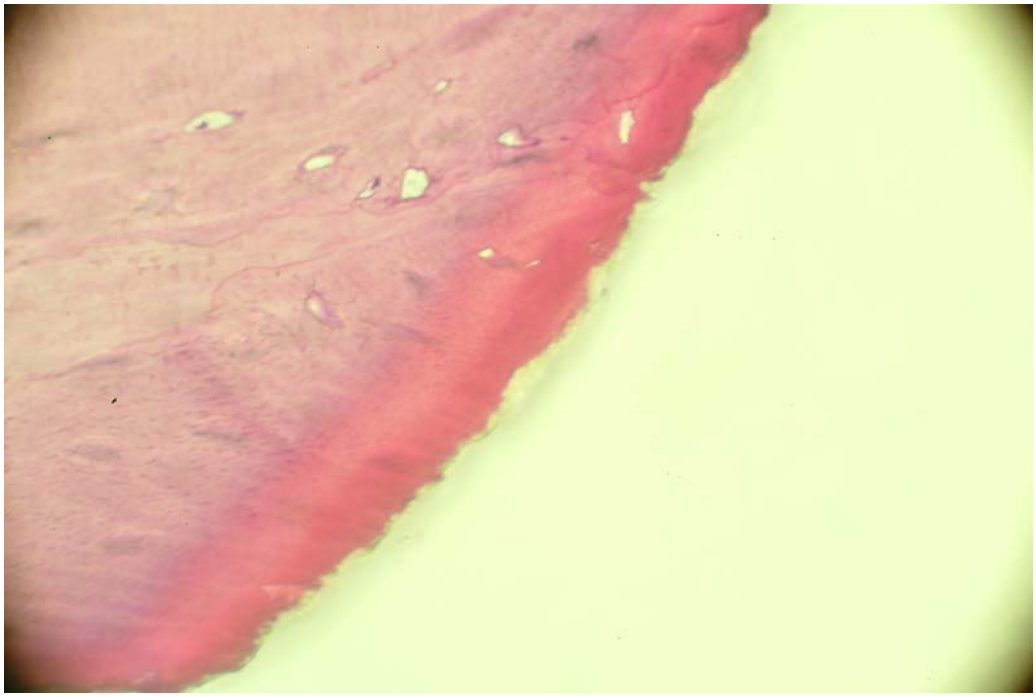


Fig. 42: A1/2 – Magnification factor 40 x

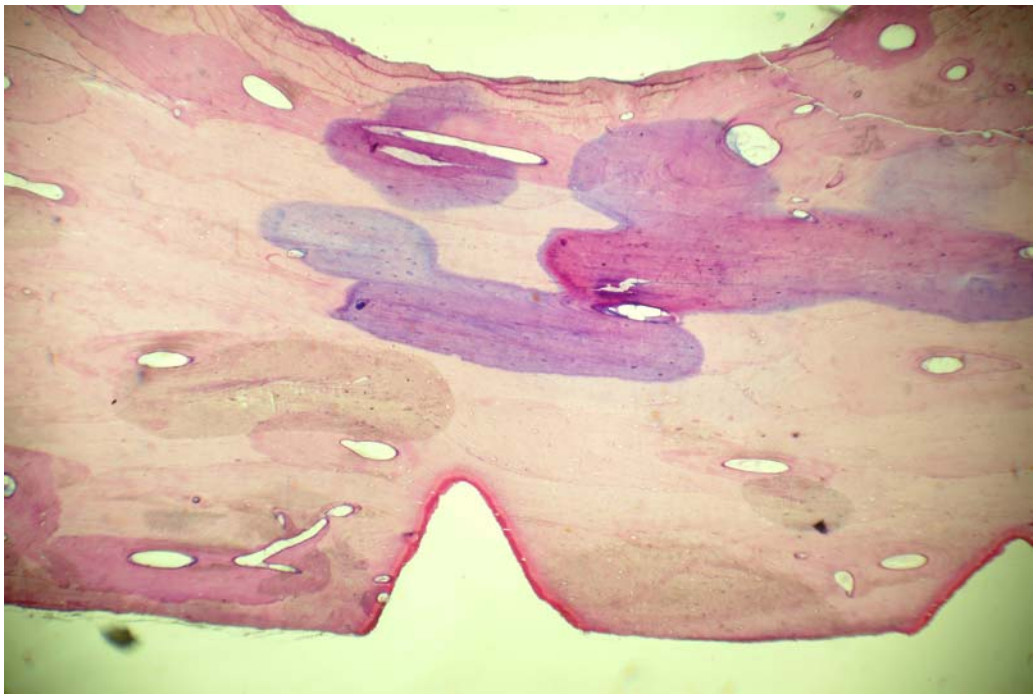


Fig. 43: A1/3 – Magnification factor 4 x

The laser beam always produces a conic window depending on absorption of energy.

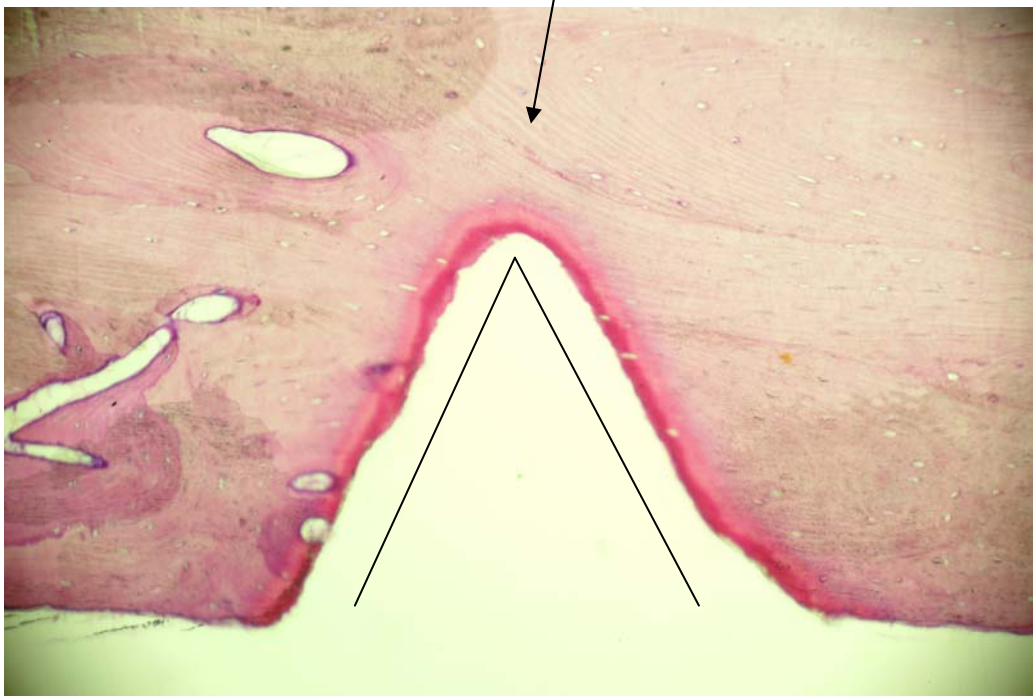


Fig. 44: A1/3 – Magnification factor 10 x (including explanation)

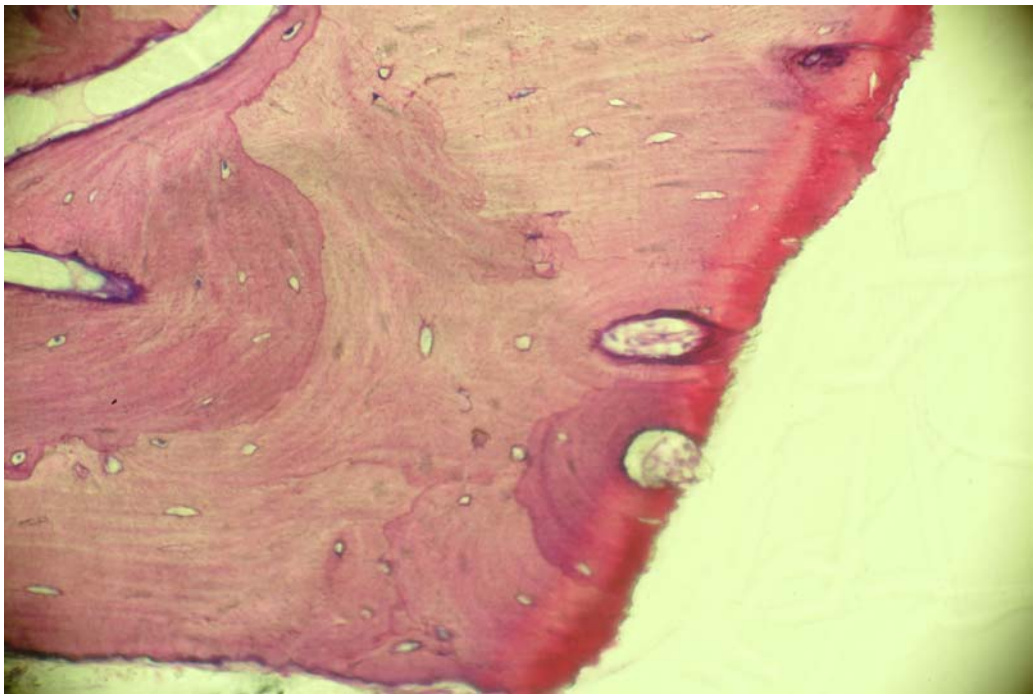


Fig. 45: A1/3 – Magnification factor 25 x

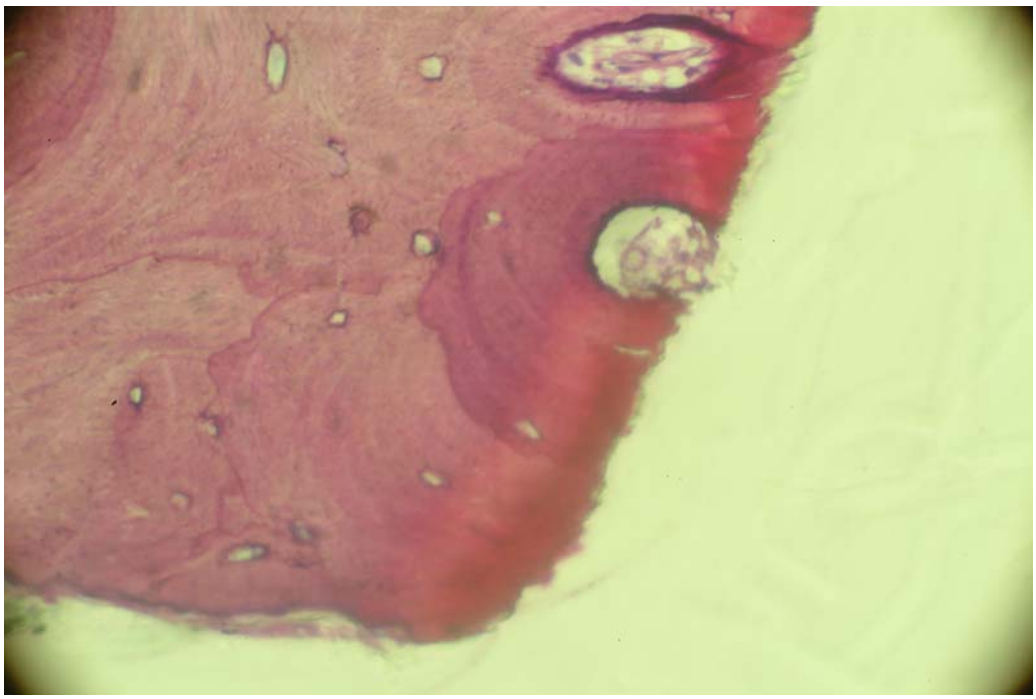


Fig. 46: A1/3 – Magnification factor 40 x

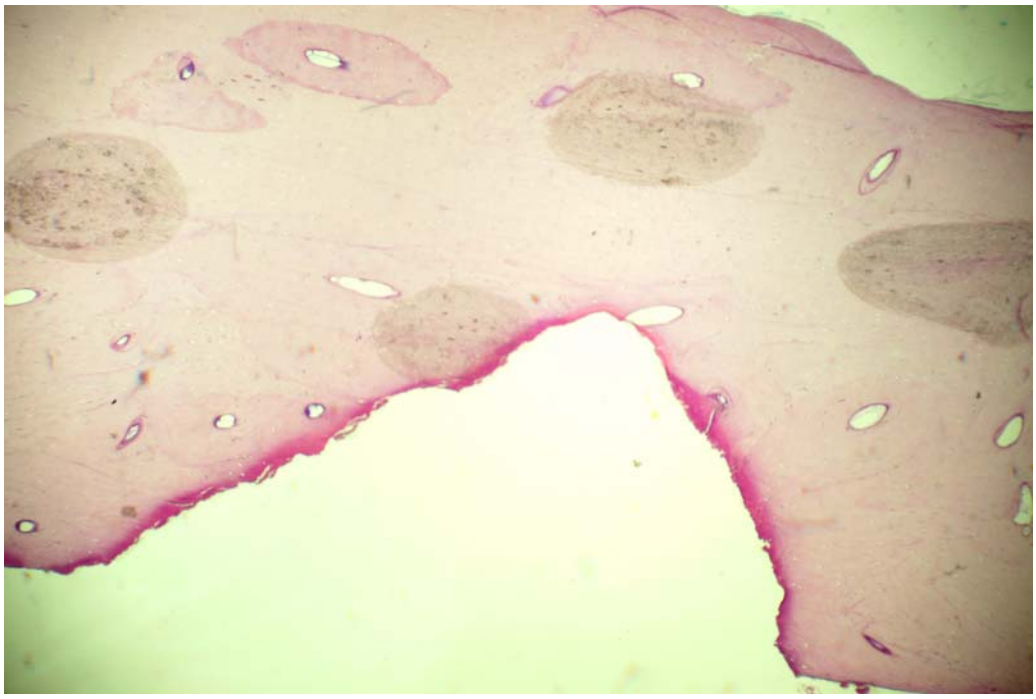


Fig. 47: A1/4 – Magnification factor 10 x

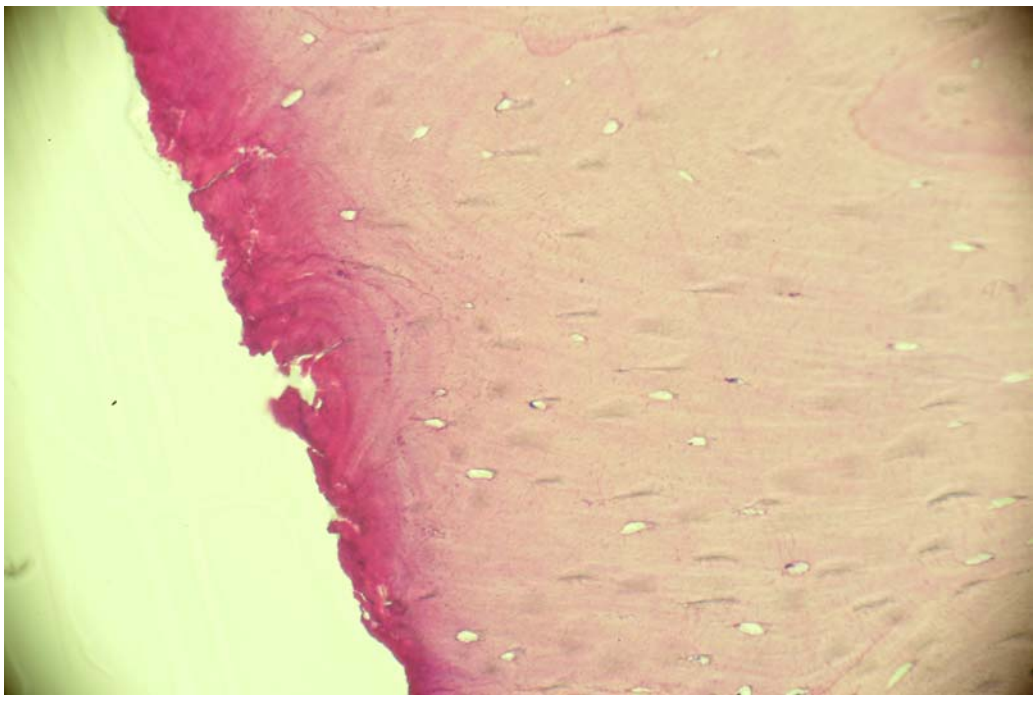


Fig. 48: A1/4 – Magnification factor 25 x

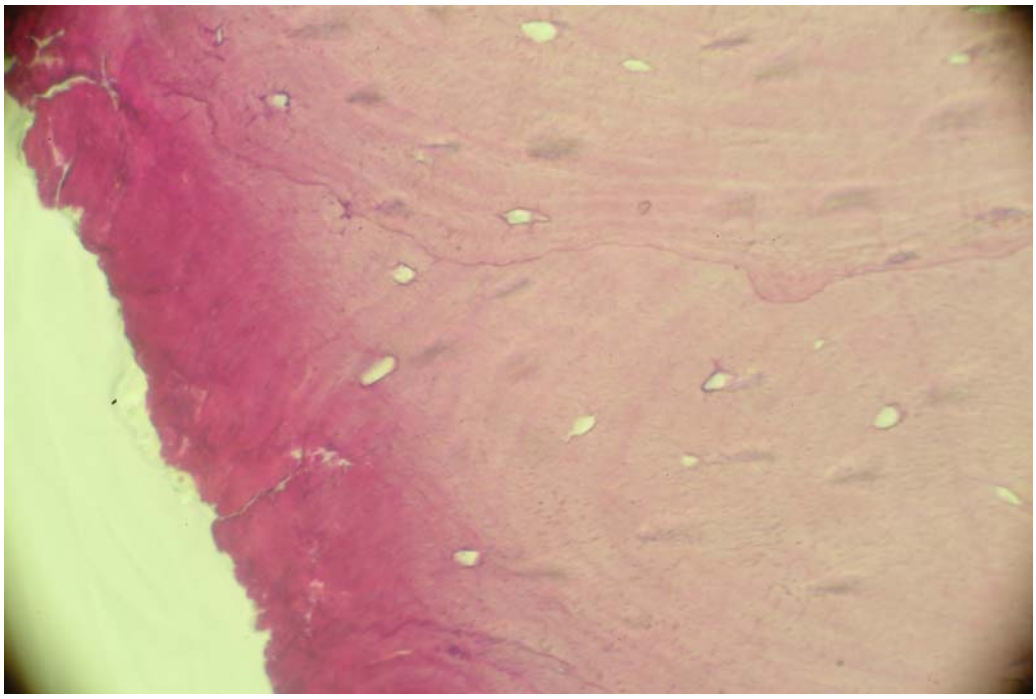
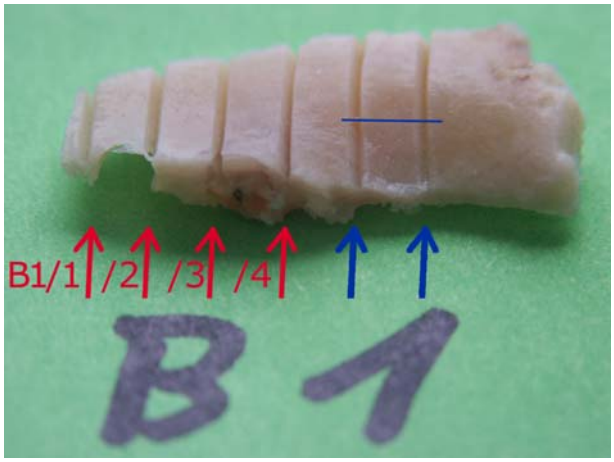


Fig. 49: A1/4 – Magnification factor 40 x

3.4.1.5 Preparation B1



The red arrows indicate the paths run, the blue arrows indicate the paths which were not included in the evaluation

Working planes 1 to 4:

Path 1 run: 1 mm in 2 sec. x 2

Path 2 run: 1 mm in 3 sec. x 2

Path 3 run: 1 mm in 4 sec. x 2

Path 4 run: 1 mm in 5 sec. x 2

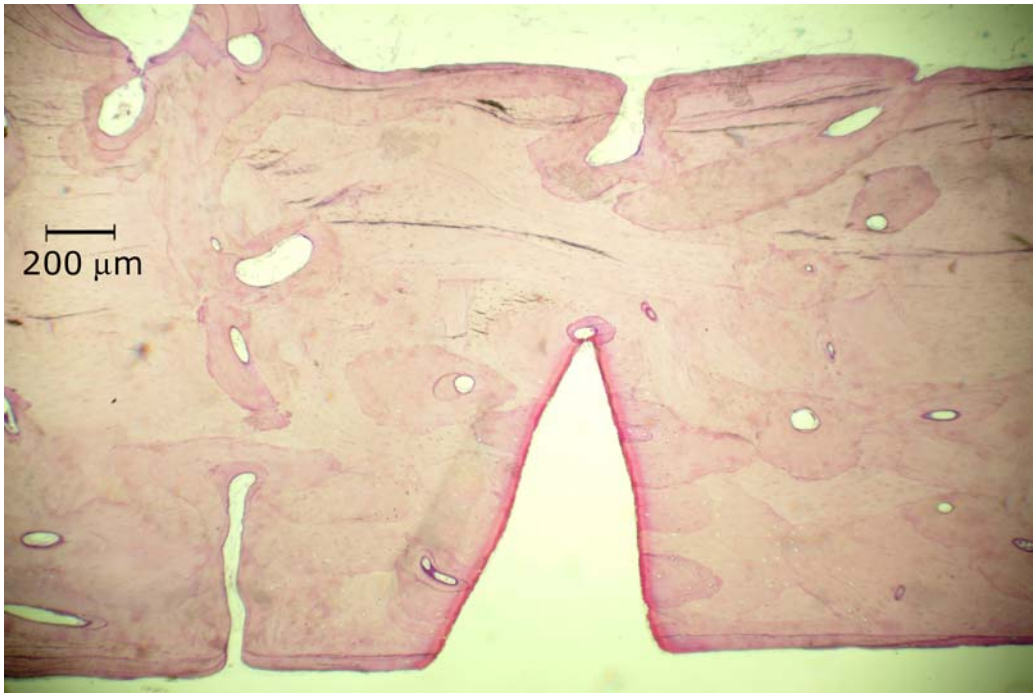


Fig. 50: B1/1 – Magnification factor 4 x

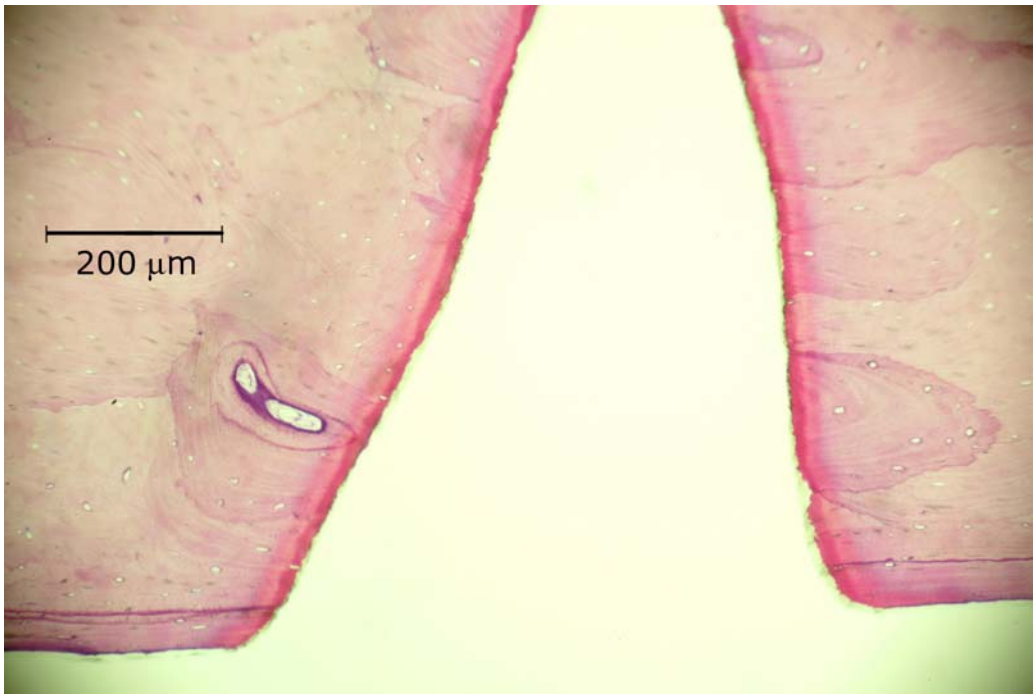


Fig. 51: B1/1 – Magnification factor 10 x

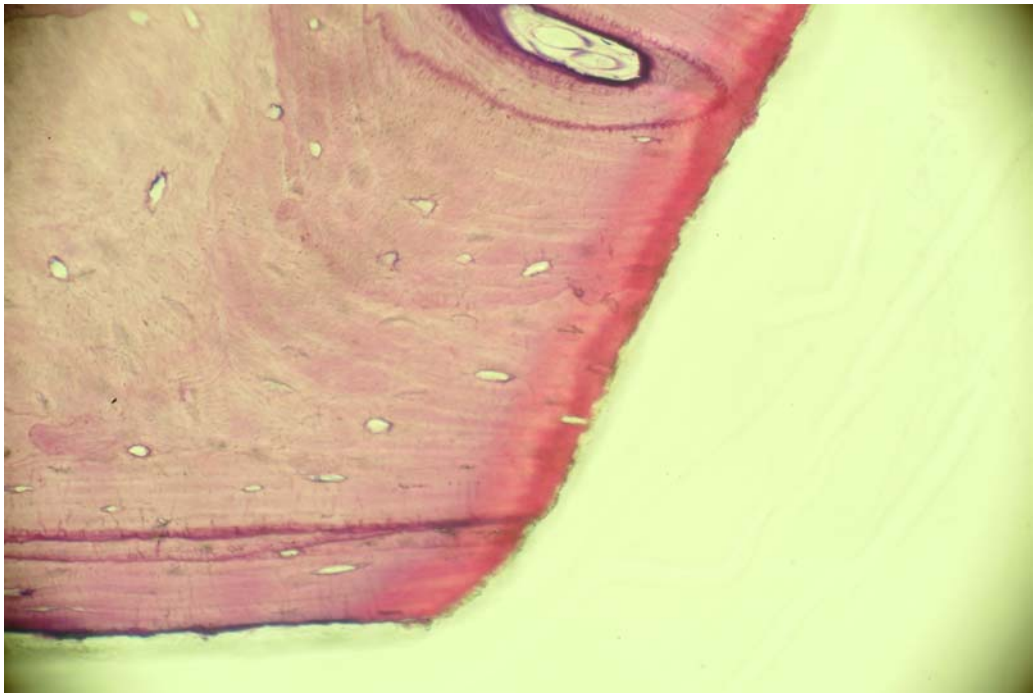


Fig. 52: B1/1 – Magnification factor 25 x

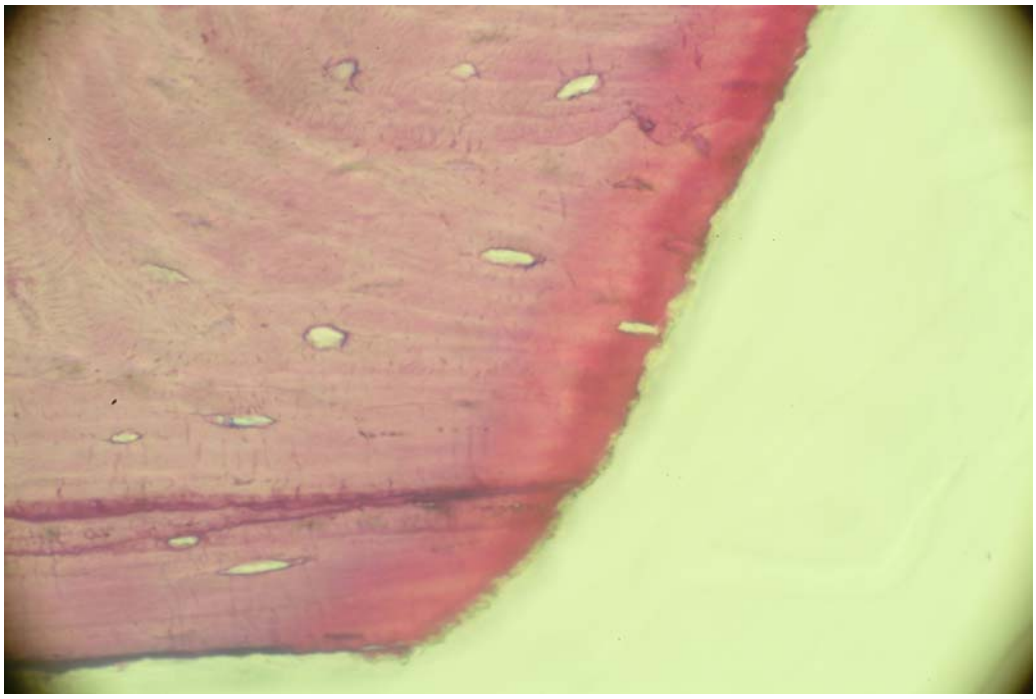


Fig. 53: B1/1 – Magnification factor 40 x

The laser beam always produces a conic window,
but during this arrangement the cone is longer and pointed.

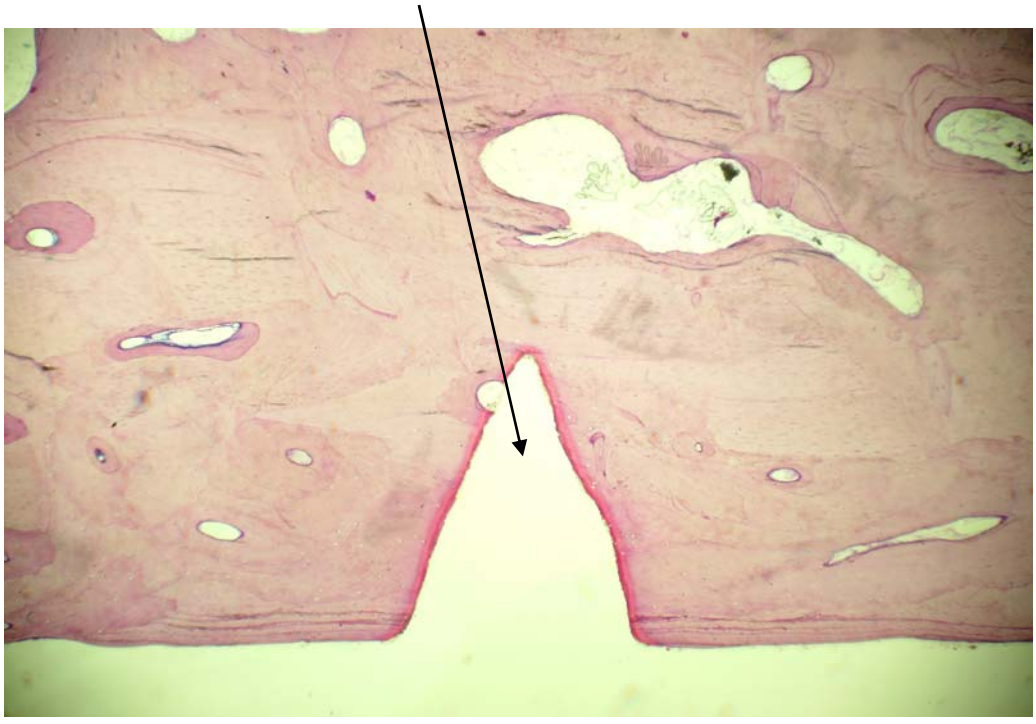


Fig. 54: B1/2 –Magnification factor 4 x (inclusive explanation)

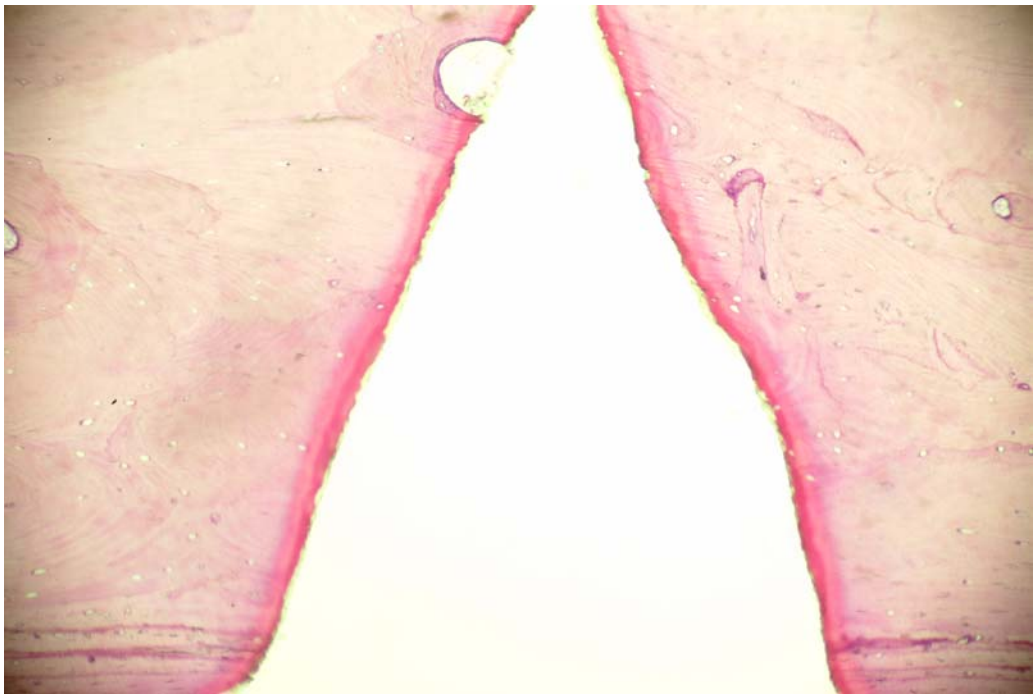


Fig. 55: B1/2 – Magnification factor 10 x

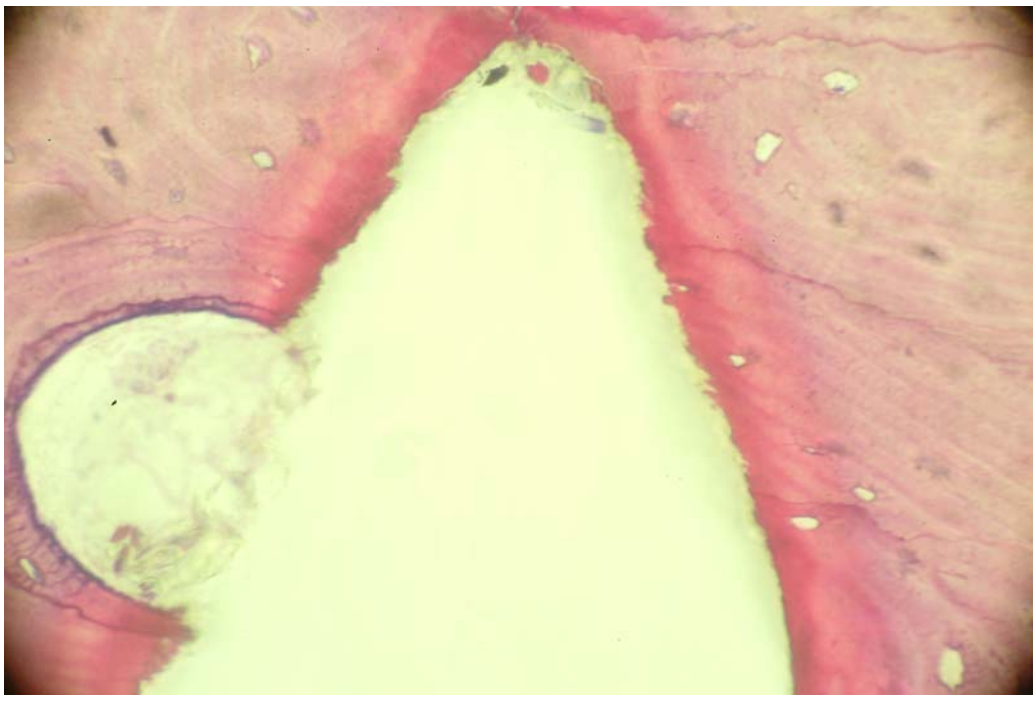
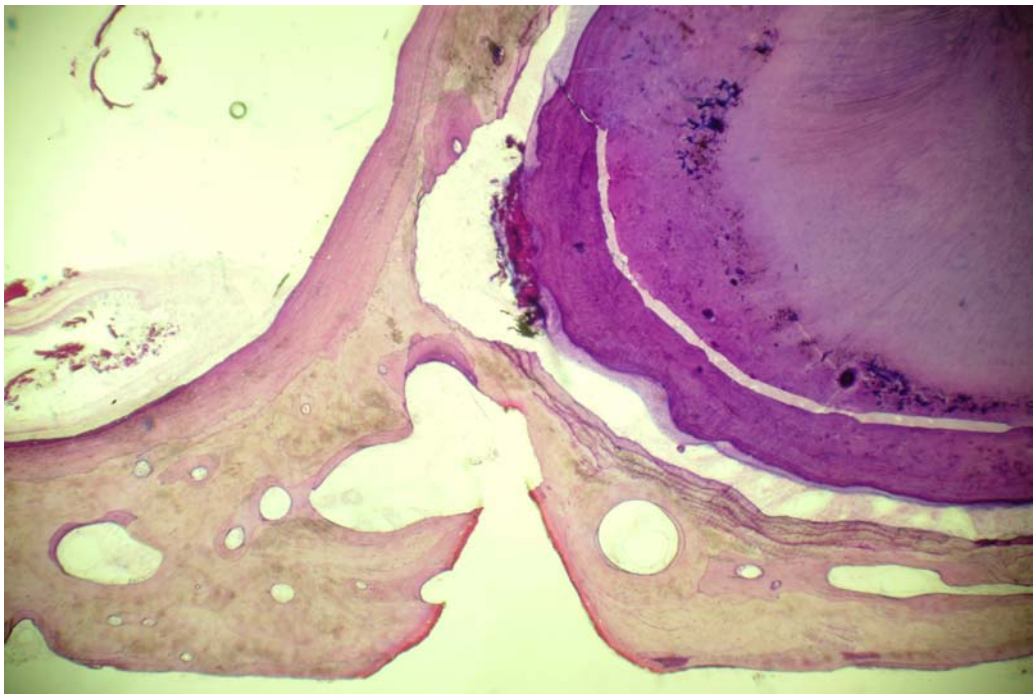


Fig. 56: B1/2 – Magnification factor 40 x



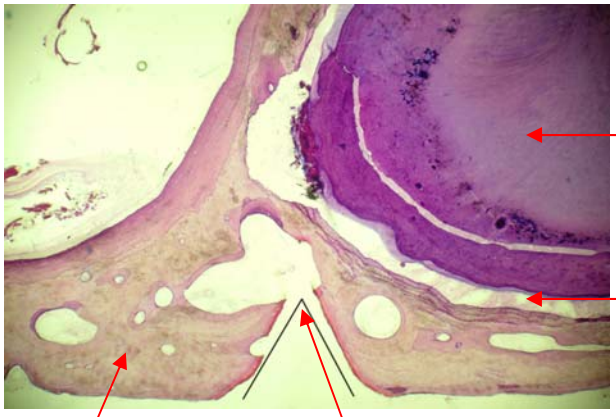


Fig. 57 a and b: B1/3 – Magnification factor 4 x (and explanation)

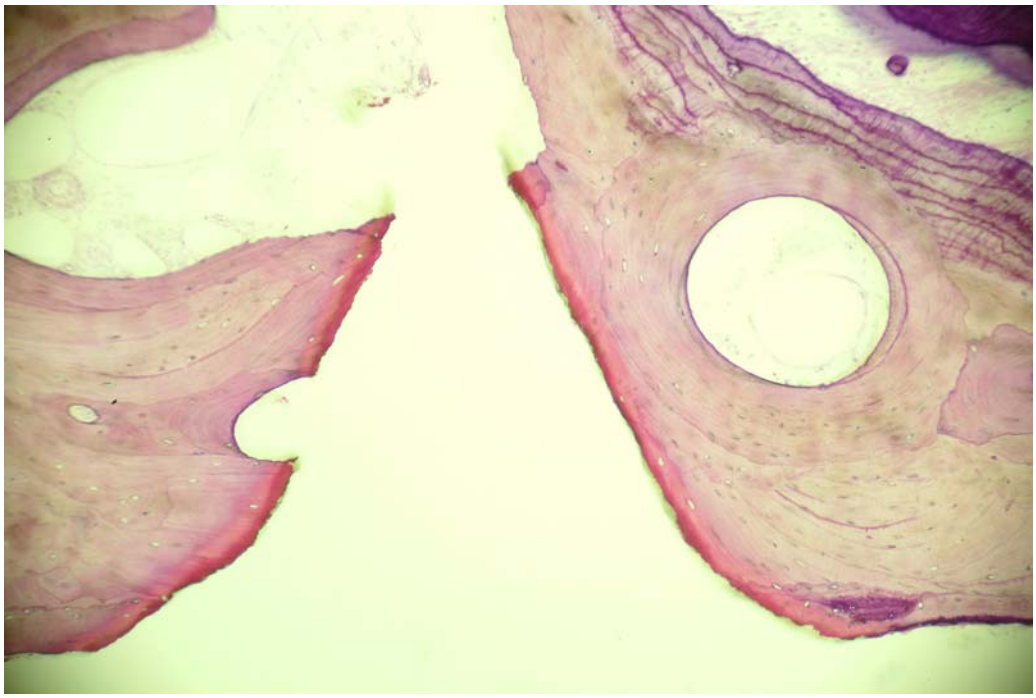


Fig. 58: B1/3 – Magnification factor 10 x

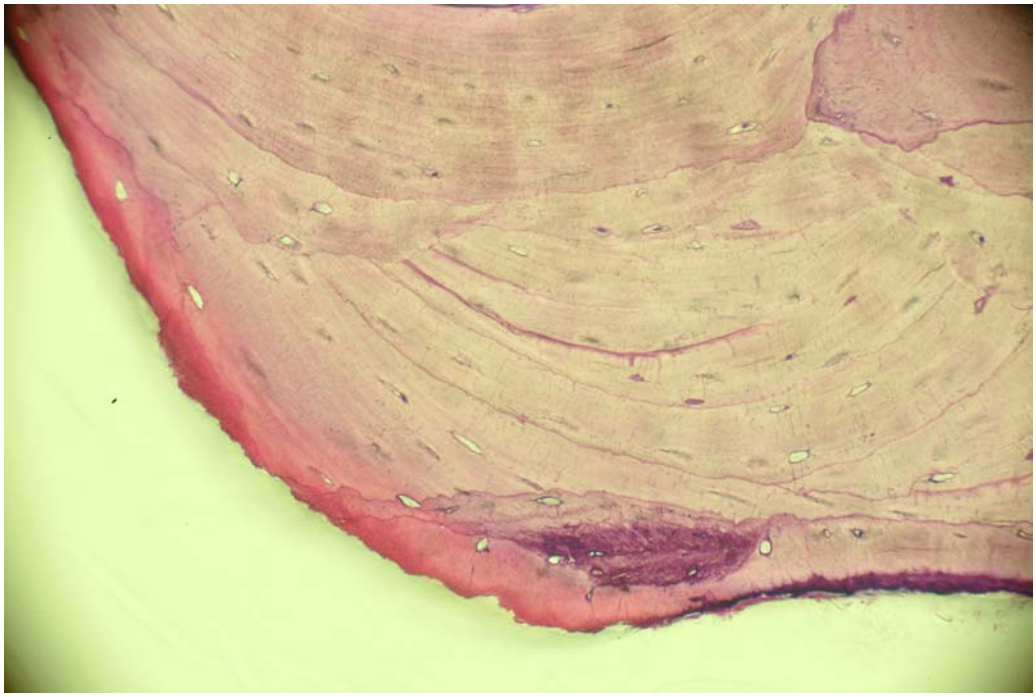


Fig. 59: B1/3 – Magnification factor 25 x

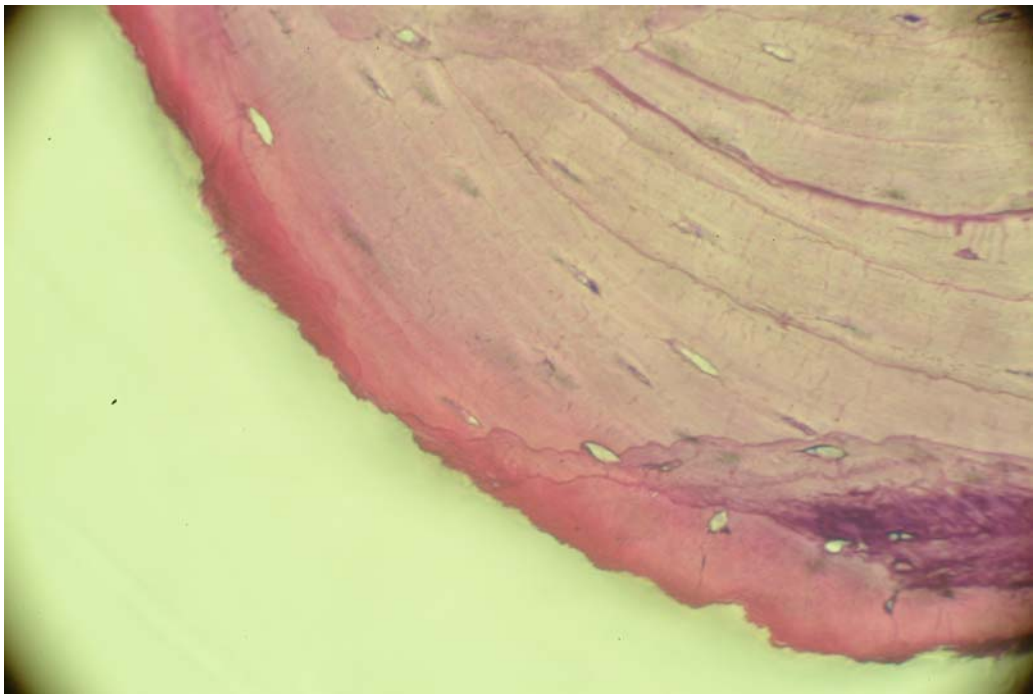


Fig. 60: B1/3 – Magnification factor 40 x

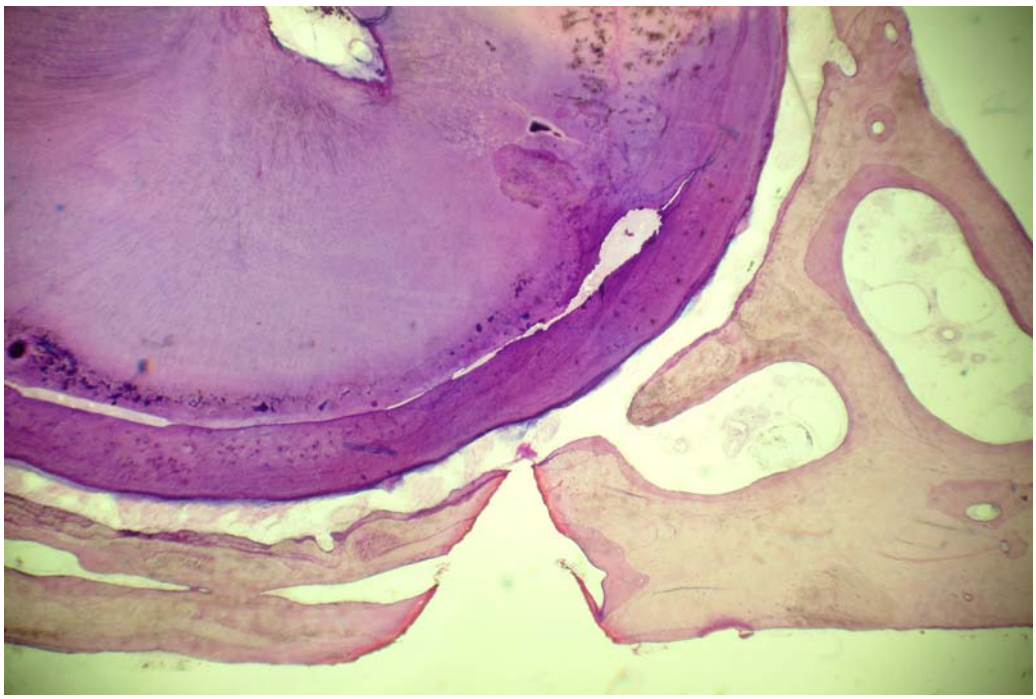


Fig. 61: B1/4 – Magnification factor 4 x

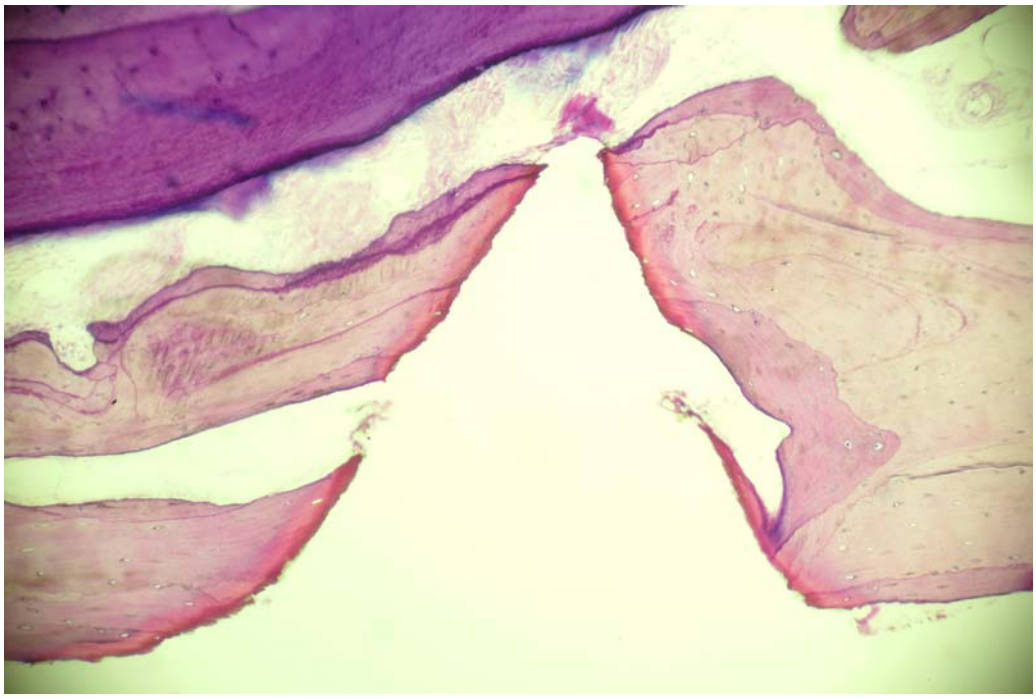


Fig. 62: B1/4 – Magnification factor 10 x

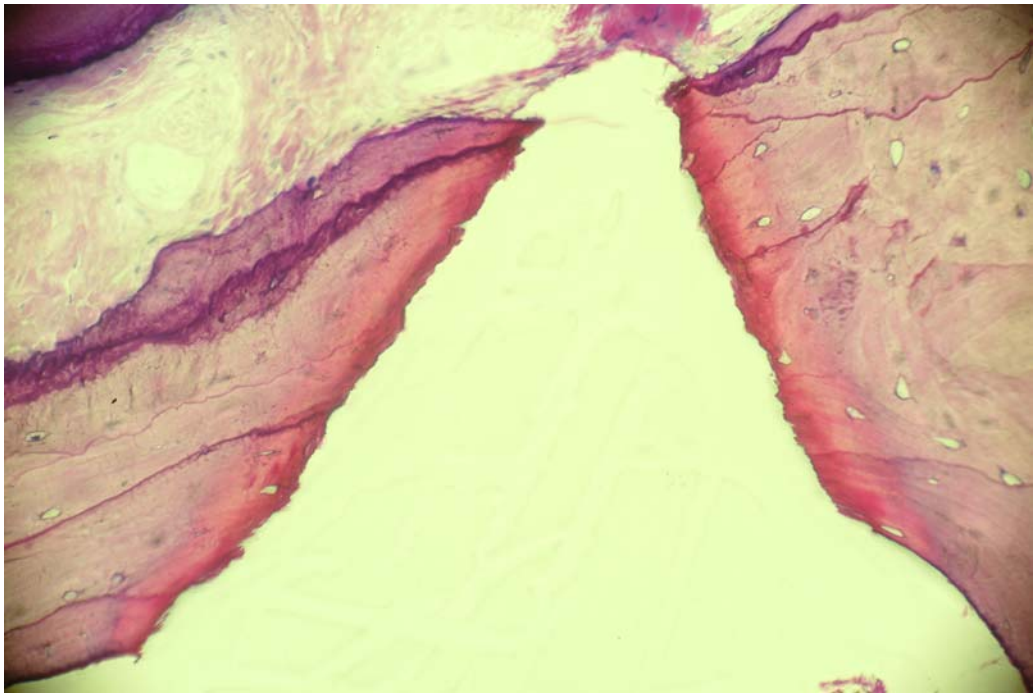
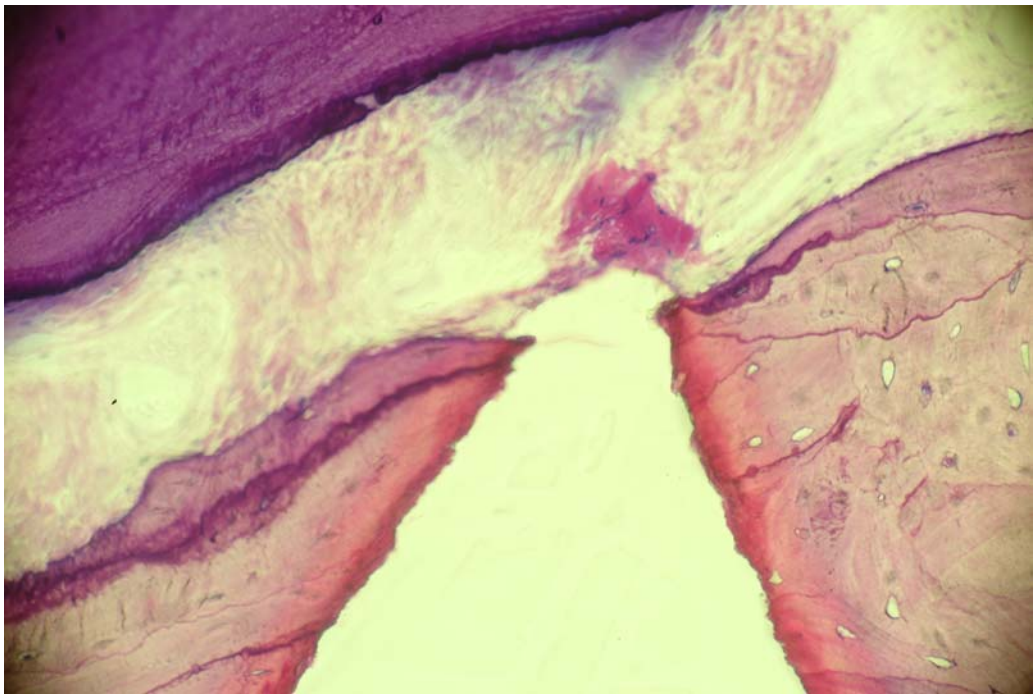


Fig. 63: B1/4 – Magnification factor 25 x



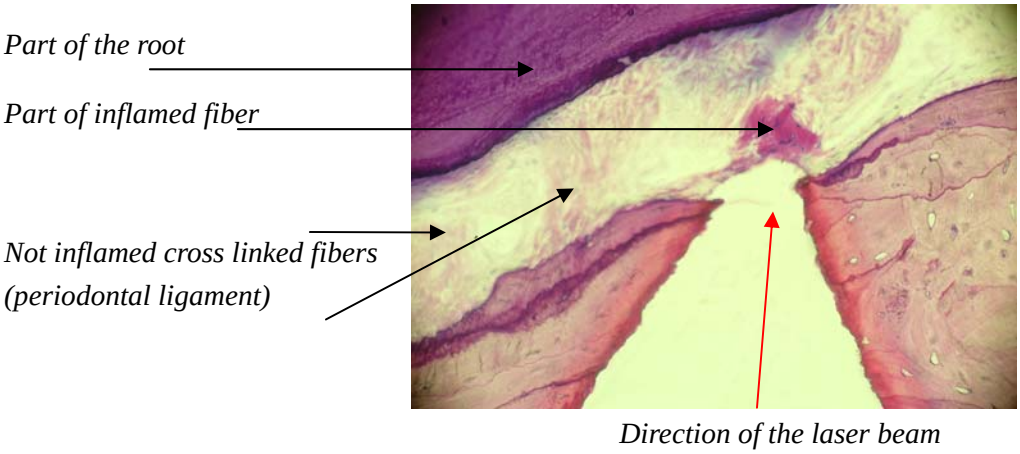


Fig. 64 a & b: B1/4 – Magnification factor 25 x (another section and explanation)

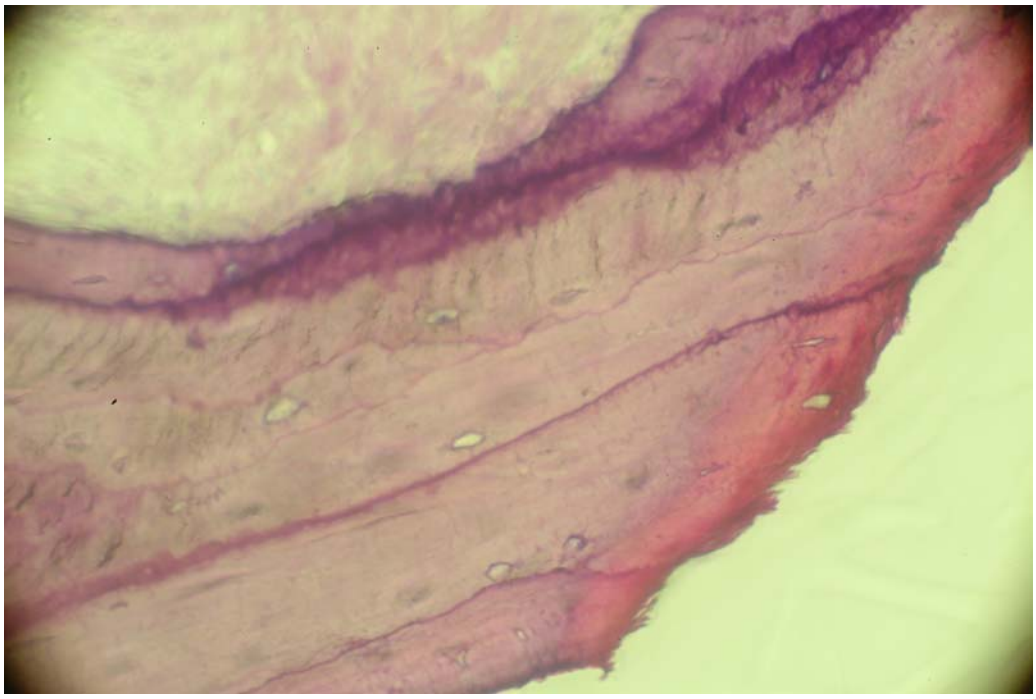


Fig. 65: B1/4 – Magnification factor 40 x

3.5 Working distance and constancy of path / time, here mm/sec

The working distance and the focus of the laser used was 3 mm (3.1.1 to 3.1.5) above the tissue; the exposure time of the laser was carried out here depending on energy, path, and time.

Path/time, here mm/sec, was never taken into consideration in any of the clinical studies up to now. Even for the motion of the laser, only the individual hand guidance of the user observed was taken (Sample in [Lit. 33]).

The histology of the corresponding preparations is equivalent. Time, path and energy have no influence on the statement of the microscopic findings (3.1.1 to 3.1.5); they are practically uniform. This is probably due to the high, uniform cooling (3.1.1 to 3.1.5, and 2.6.4) and precise focusability of the laser beam.

The macroscopy and the microscopy show no clear allocation, e.g. for the use of Tip G6 (600 µm, calibration factor 1.0). The working depth was not doubled when the working time of the laser was increased from 1 mm/sec to 1 mm in 2 sec (3.4.1.4 and 3.4.1.5).

The laser cut width is always in the range of 720 µm. This, of course, also applies to doubly processed paths, such as in preparation B1 (3.4.1.5). This shows clearly how important it is always to work in focus. Magnifying spectacles should always be worn to observe and correct the procedure as necessary.

The inflamed zones in the bone (Fig. 38) are also uniform, regardless of path, time and energy. The statistical evaluation provides no significant reference value. An earlier statement of mine (lecture to WCLI Congress 2007 in Los Angeles) regarding carbonization on the preparation has to be contradicted; according to the latest measurements this is only debris, to an extent of no more than 6 µm.

The following zone (Fig. 39) referred to as necrosis is actually uniform in all preparation views, i.e. ca. 20 µm wide. Although the anatomic structures here are only partially altered, I still described them as necrosis. This has a lining of a different color (Fig. 38) as an area inflammation with somewhat fluctuating dimensions between 20 and 30 µm which could be described as an inflamed reversible zone. If histocytes or erythrocytes can be detected, their cell walls and nuclei are not lysed.

If we observe the preparation B1, path 4 (Fig. 63 and Fig. 64), we can see that here the laser was working on two different tissues. We can see that the laser shot a funnel-shaped crater through the bone and only left a small inflammation zone shown in color in the peridontal ligament. We can also see here that, despite a high energy density of 2 x 17,500 mW/mm (Table 10), there is no further thermal damage. Ultimately it can again be established that the laser must be guided exactly in focus in order to have an effect; with spray cooling no further thermal damage can be expected outside of the focus.

The shape of the shot window produced by the Er,Cr:YSGG laser is clearly rounded at the deepest point in the single processed paths of the preparation group A1 to A4 (3.4.1.1 to 3.4.1.4). In preparation B1 (3.4.1.5) with a doubly processed path, the shot funnel is more conical in shape and ends in a point.

It should also be noted that in the preparation group A1 to A4 (3.4.1.1 to 3.4.1.4) the entry angle of the laser is square, while the exit angle is rounded (Fig. 36). In preparation group B1 (3.4.1.5) the entry angle also appears rounded. This means that the entry angle in the second path must have been slightly within the focus. As in the other histological images debris can be clearly seen at the end of the shot tunnel in Fig. 56. This was removed constantly during processing with the spray cooling. This is where spray cooling of approx. 0.8 ml/sec finds its most important application: If the debris is not sufficiently diluted and removed, this can cause burns at the laser tip and uncontrolled thermal effects on the tissue being treated.

4. Discussion

It was possible to verify that even in the case of extreme energy values (Table 10), at exact parameters (Table 1, Table 3 to Table 10) the Er,Cr:YSGG laser by the Biolase company generates no carbonization or significant necrosis. Working under clinical conditions it is noticed that during the processing of the bone and of the soft tissue, the blood flow is never interrupted, so that healing is always ensured. I have also experienced this in practice. Due to the test assembly described, the effects of the laser application under concrete, reproducible parameters are clearly documented. Although the corresponding literature in the area of “lasers in endodontics” includes far more defined parameters, soft tissue and, in particular, hard tissue are only described with individually hand-guided parameters. These completely ignore the ratio of energy to time and path at a controlled distance. It is unimportant here whether we are talking about tests on the effects of the Er,Cr:YSGG laser [Lit. 3, 6, 15, 17, 21, 26, 32, 35, 36, 38] or about the description of the effect of the Er:YAG laser [Lit. 9, 10, 13, 18, 27, 33, 34]. As the Er,Cr:YSGG laser and the Er:YAG are to a certain extent competing on the dental market, the working values are naturally somewhat contentious which can also lead to a certain degree of polemics.

In the literature [33, 34] it is claimed, among other things, for an Er:YAG laser that bones at up to 15 Watt and 5 to 20 Hz are only cooled with 6 ml H_2O/min of water/air spray. The cooling values discussed here for the Er,Cr:YSGG laser are within the certain range of approx. 42 ml H_2O/min of water/air spray. At the very low frequency difference of 2,780 nm to 2,940 nm, i.e. 10 %, and not, as described, to the power of ten [Lit. 34], the values stated for cooling appear too low. The stated histology [Lit. 33] is not based on sound criteria; a direct comparison is not possible anyway due to the absence of parameters such as path and time. The working distance of 8 to 12 mm with a focusing range of 4 mm (Er:YAG Laser) appears too variable and cannot be precisely maintained without aids. In practice distal application to tooth 6 at these distances is presumably hardly possible. A pig’s rib has to serve the purpose here, as described in the study [Lit. 33]. Nor is there any mention that the work was carried out

wearing magnifying spectacles so that small tactile laser handpiece maneuvers can be carried out. Today magnifying spectacles are regarded as an indispensable instrument for minimally invasive treatment.

The stated spot radius of 250 to 290 µm [Lit. 33] does not stand up to comparison with that of the cut image in the rib; the cut width is more like three times the size. The statement that the increase of the pulse energy to 1,000 mJ does not damage the bone contradicts the histological image used to show this [Lit. 33]. The zone of inflammation resulting from the exposure to heat which must always be represented in histological images cannot be seen in the cited pictures, nor is it discussed. There simply cannot be a seamless zone from necrosis to healthy tissue. The exact spray cooling stated by the author of the cited study [Lit. 33] is actually not known in this form. If the spot size (cut width) stated in the cited study [Lit. 33] of 290 µm and 5 to 6 mm cut depth at 6 ml/min spray cooling is observed, one can expect that this will clearly lead to a build-up of heat in the cut depth. Carbonizations and necrosis zone would be significantly enlarged. The statement in the cited study of 6 ml/min at 3.5 bar compressed air does not suggest a clear spray volume. Thus the statement of 3.5 bar is irrelevant here. The table [Lit. 33] of the values 500 mJ, 750 mJ and 1,000 mJ at 5, 10, 15, 20 Hz states measured values of a maximum of 15 to 12 to 11,5 µm and describes the last value at 1000 mJ as the lowest for the generation of necrosis. Furthermore, the Er:YAG laser is described ablatively and loses the capability of actual disruption. The values prescribed for a cold ablation of 10^4 to 10^9 Watt/cm² are not reached here.

In short, the trial on the effect of the Er,Cr:YSGG on bone shows that with the correct cooling, regardless of the energy application and the focus, the hardly definable width of the necrosis remains unchanged. This can also be assumed for the soft tissue and certainly shows better values compared with the tests [Lit. 34]. This would, however, have to be verified by a further study. A pulse width, even in comparison with the Er:YAG [Lit. 33, 34], which could be adjusted as a variable, appears nowhere in the discussion for the Waterlase.

The aim is a minimum pulse width with steeply rising and falling application of energy in order to reduce the thermal load with the best possible disruptive efficiency in the tissue.

The dermatological applications of the Er:YAG with its thermal side effects are not relevant here to the debate on dental applications and serve no purpose. In addition to this, the zone with the inflammation must also be counted as thermal alteration, as this zone first has to recover in order to ultimately overcome the necrosis zone and to allow a contact healing of the cut areas.

In conclusion, the advantages of the use of the Er,Cr:YSGG laser are clearly obvious.

5. Summary / Zusammenfassung

5.1 Summary

This thesis describes for the first time the effect of the Er,Cr:YSGG laser, type Millennium, on human bone tissue, in particular taking into account the factors of continuous focus, path and time. The registration of these factors was possible because the laser handpiece was guided by machine-aided, reproducible parameters over the tissue. So far there are no comparative studies on this study. The device developed for this study substituted the imprecise, individual hand-guided method, which can supply no comparable values.

The histological evaluation shows that with sufficient cooling, constant focus and energy outputs of the laser of up to 5 Watt, even with longer time exposure per measured length of path, no carbonization could be generated. What is most important with this laser is the perfect cooling spray of water and air, which is only achieved by this laser.

The laser causes a wedge shaped cut in the bone. The definite zone of the debris amounts to approx. 6 µm. The zone of the assume necrosis amounted on average to ca. 20 µm, that of the reversible inflammation a width of 20 to 30 µm.

With the cooling by water spray used here and a working angle of 25° it was possible to verify that the application of the Er,Cr:YSGG laser on human bone can be regarded as safe. Due to the low focus distance to the bone, this laser is also suitable for use in the narrow confines of the oral area, particularly where, according to the literature, the Er:YAG-Laser with necrosis zones and ca. 3 x working distance cannot be used or only with great difficulty.

5.2 Zusammenfassung

Die vorliegende Arbeit beschreibt erstmalig die Einwirkung des Er,Cr:YSGG-Lasers, Typ Millennium auf humanes Knochengewebe, insbesondere auch unter Berücksichtigung der Faktoren kontinuierlicher Focus, Weg und Zeit. Die Erfassung dieser Faktoren war möglich, weil das Laserhandstück mittels maschinengeführter, wiederholbarer Parameter über das Gewebe geführt

wurde. Bisher gibt es zu dieser Studie keine Vergleichsstudien. Das für diese Studie entwickelte Gerät ersetzte die nicht exakte individuelle handgeführte Methode, die keine vergleichbaren Werte liefern kann.

Die histologische Auswertung zeigt, dass bei ausreichender Kühlung gleich bleibendem Focus und Energieleistungen des Lasers bis zu 5 Watt, auch unter hoher zeitlicher Einwirkung pro gemessener Wegstrecke keine Karbonisierungen erzeugt werden konnten. Das wichtigste für diesen Laser ist die perfekte Spraykühlung aus Wasser und Luft, die nur mit diesem Laser erreicht wird.

Der Laser bewirkt einen keilförmigen Schnitt im Knochen. Die definierte Zone der Zelltrümmer[14] beträgt etwa 6 µm. Die Zone der angenommenen Necrose betrug im Mittel ca. 20 µm, die der reversiblen Entzündung eine Breite von 20 bis 30 µm.

Unter Berücksichtigung der hier angegebenen Kühlung durch Wasserspray und Arbeitswinkel von 25° konnte nachgewiesen werden, dass die Anwendung des Er,Cr:YSGG-Lasers am humanen Knochen als gesichert angesehen werden kann. Aufgrund des geringen Focusabstandes zum Knochen eignet sich dieser Laser auch im engen Zugangsbereich des oralen Bereiches ganz besonders dort, wo der Er:YAG-Laser laut Literatur mit Necrose-Zonen und circa 3-fachem Arbeitsabstand nicht oder nur erschwert anzuwenden ist.

[14] Debris

6. Acknowledgement

At this point I would like to thank Mr. Professor Dr. med. Klaus-U. Benner, Anatomic Institute of the Ludwig Maximilian University of Munich, Germany for the discussion, materials, and histological investigations.

I would also like to thank Ms. Monika Droeger for the graphic and written production of this work.

7. Appendix

7.1 Table of figures

7.2 List of Tables and Protocols

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9. Assurance

All results and pictures are reported without any manipulation. The studies are carried out without sponsoring of any company.

Berlin, 28.08.2007

Harald Passow

10. Curriculum Vitae

Name: Harald Passow

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Education and career

1957 – 1964 Primary school

1964 – 1966 Vocational school. Training in the vocational area of agricultural and fisheries management.

1966 – 1967 Attendance at the Regional Institute for fisheries of the Land of North Rhine Westphalia (Landesanstalt für Fischerei des Landes Nordrhein-Westfalen) for further training First vocational qualification as fish breeding assistant.

1967 – 1969 Training at the same regional institute as a biological-technical assistant with final examination in the area of pathology in freshwater fish.

1969 – 1970 Employed as a biological-technical assistant with the company Farbwerke Hoechst AG in Frankfurt/Main, Department for Analytical Chemistry;
Residues analysis in the field of insecticides, Dr. Kelker.

1970 – 1971 Employed as a biological-technical assistant at the Free University of Berlin in the Pathology Institute as a technical lab director in the field of hormone research with Gastrin I and II, Department of Dr. H.-P. Seelig.
Co-author of two scientific publications.

1972 – 1974 Employed as a biological-technical assistant at the Max Planck Institute for Molecular Genetics, Department of Prof. Dr. H. G. Wittmann. Worked in the examination of the structures and functions of the ribosomes.

Extended working period at the University of Chicago.
Co-author of a scientific publication.

1970 – 1974	Attendance at evening school for the intermediate certificate and the general matriculation standard certificate..
1975 – 1977	Employed as a biological-technical assistant at the Free University of Berlin, Institute for Molecular Biology and Biochemistry, Department of Prof. Dr. H.-J. Risse. Worked in the area of cell differentiations, here on the model organism Dictyostelium discoideum. Co-author of scientific publications.
1978 – 1978	Brief period managing my parents' fish-breeding plant for family reasons.
1979 – 1984	Study of dental medicine at the Free University of Berlin in the regular study period graduating as a dentist.
1984 – 1986	Work as an assistant in various dental practices in Berlin.
1987	Opened own dental practice in Berlin-Zehlendorf.
1994 – 1995	Further training with qualification as a dentist for natural healing.
1999 – 2000	Training with qualification curriculum Implantology of the DGZI (Deutsche Gesellschaft für Zahnärztliche Implantologie – German Institute for Dental Implantology).
2005	Achievement of the M.Sc. in Implantology with the master thesis "Design of a standard test assembly for controlled laser studies on tissue. Initial studies and results on human bone material" at the Danube University of Krems, Austria.
2005	Matriculation as a mature student at the Danube University of Krems to achieve the Master of Science in Oral Surgery"; expected graduation in 2007.

2005	Doctorate to achieve the degree of Dr. med. dent. on the subject of “Rehabilitation in the case of malignomes in the ENT area. Rehabilitee structure, rehabilitation requirements and rehabilitation results” at the Johann Wolfgang Goethe University of Frankfurt am Main.
2006	Module studies I and II and qualification as a specialist in” oral laser medicine” at the SOLA in Vienna.