
Preparation of Histologic Sections

by the Cutting-Grinding Technique
for Hard Tissue and other Material
not suitable to be sectioned by routine methods

– Equipment and Methodical Performance –

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The cutting-grinding technique is a method used to obtain thin sections (below 10 μm) for histological examination of specimens which cannot be cut by conventional techniques (Fig. 5). These include such tissues as jaw bones with teeth containing fillings, crowns and bridges, thick cortical bones, implants (metals or ceramics) jaw or long bones or even brittle hypermineralized bone parts.

One can see from the proceeding list of specimens that the cutting-grinding technique is not a substitute for the conventional Cryo-paraffin and hard cutting techniques because most of these tissues cannot be manipulated by the paraffin technique after decalcification. The solution for this problem has been approached in different ways. GROB and STRUNZ (1977) presented their findings using surface staining on "saw cuts" 50–200 μm thick. Others followed these saw cuts with mechanical or manual grinding to reduce the thickness to 30 μm (HENSCHKE and PESCH, 1980 et al).

The method to be described here (DONATH and BREUNER, 1982) is routinely used in daily histological diagnosis for specimens which cannot be processed in paraffin. Numerous innovations have been developed through the routine use of this method. These include not only new equipment but new staining methods and the use of a plastic resin for embedding the specimen. To prepare thin slices for histologic diagnosis by transillumination the following machines, accessories and supplies are needed.

Machines

- 1.1 Exakt-cutting-grinding system
- 2.2 Exakt-micro-grinding system

Accessories

- 2.1 Exakt-vacuum-adhesive system device for preparing parallel-sided blocks
- 2.2 Exakt-precision-adhesive press for mounting the plexiglass slides
- 2.3 Exakt-light-polymerization unit
- 2.4 Exakt-dehydration- and infiltration-unit
- 2.5 Exakt-block-drying, -infiltration, -polymerization
- 2.6 Micrometer screw with digital display
- 2.7 Feeler gauge 0,05 to 0,50 mm
- 2.8 Straight edge

1. Kulzer-Exakt:
 - Embedding medium (Technovit 7.200 VLC)
 - Technovit 4000
 - Technovit light hardening adhesive
 - Fixation adhesive (Technovit 7.230 VLC)
 - Embedding-molds (transparent)
 - Plexiglass slides
 - Abrasive paper (different grits)
2. Others:
 - Glucolmethacrylate
 - Alcohol
 - Mounting media as used in histology
 - Cover slips by Technovit 7.200 VLC
 - Benzine cleaning solution

1.1 Exakt-cutting-grinding system (Fig. 1):

The Exakt-cutting-grinding system consists of the cutting unit which is based on the principle of a handsaw, the precision-parallelizing guide with an attachment to hold the specimen to be cut and the cooling and flushing system. The speed of the cutting unit can be adjusted according to the hardness of the material in order to avoid the generation of the heat. The cutting bands are stainless-steel bands, with a thickness of 0.1 or 0.2 mm. The cutting edge of the sawblade is impregnated with diamond or boronitrid particles. Particle sizes of D 30, D 46, D 64 and D 91 are available. The cutting waste depends on the steel-band and the grit size; with a particle size of D 64 approximately 0.250 mm is lost. The precision parallelizing guide is based on the principle of a carriage which is moved behind the sawblade by an adjustable weight. In the center of the parallelizing guide is an adjusting screw system which can attach a support plate with a mechanical clamp or a vacuum plate. In the screw clamp unit a microscrew is arranged, which provides the object being moved either toward or away from the sawband with 0.02 mm increments. The cooling-flushing system consists of two water jets, one of which is positioned above the object to be cut, the other one below in or-

der to clean the sawblade. The water jet for the cooling system can be regulated.

Mechanical Operation

The samples are placed on the cutting table and held there by a mechanical clamp. The table is moved toward the sawblade by weight in a way that the specimen is cut by the diamond blade in a vertical plane. Parallel slices of selected thickness can be obtained from the tissue specimen, by advancing the specimen table in a perpendicular direction to the cutting plane, following each cut. The debris that accumulates in the chip space between the diamond bits, during the sawing procedure are moved into the flushing system where it is flushed away via a jet of water. The diamond bits eventually become clogged because of debris which developed during the process of cutting the hard plastic material. Therefore, a rough or irregular surface occurs instead of a smooth one. The cutting-grinding technique requires a plastic material of extremely hard nature. The plastic used for the hard-cut technique is elastic and therefore eventually clogs up the chip space on the diamond plate, which causes an uneven surface on the cut.

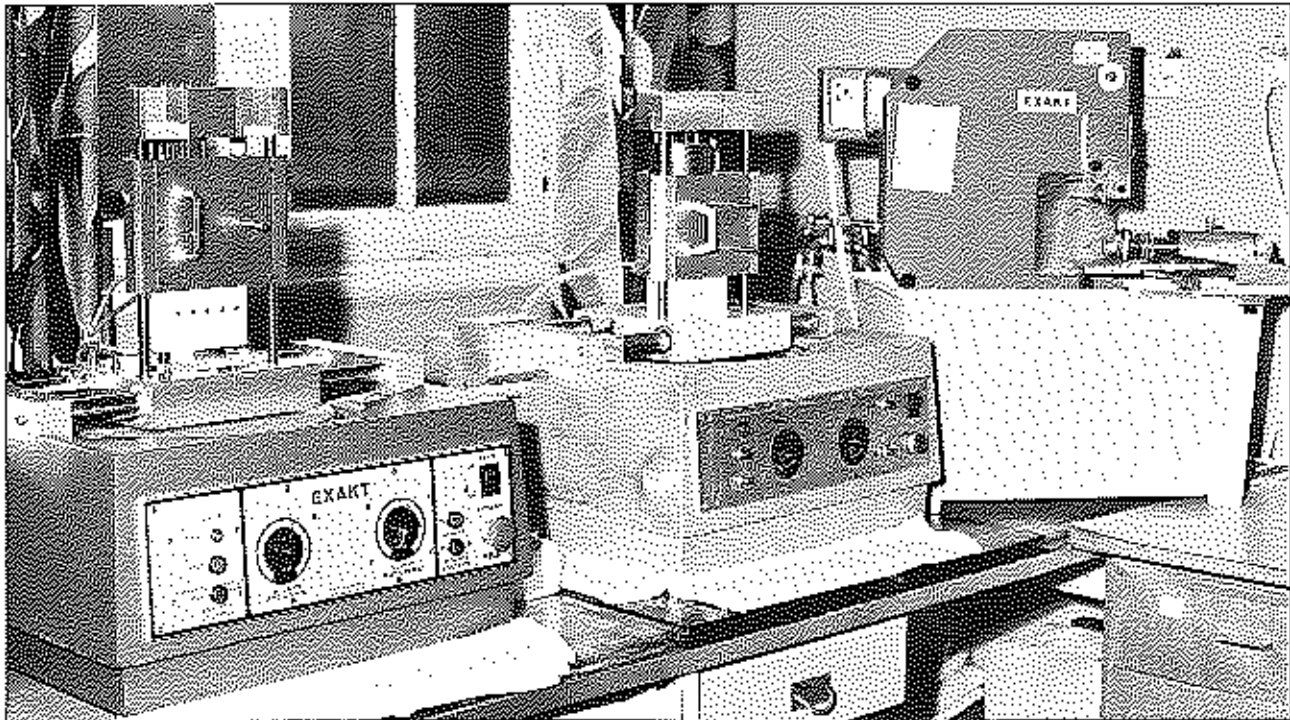


Fig. 1: View of part of Laboratory showing the Exakt-cutting grinding system (right side in the picture) and 2 machines of the Exakt-micro grinding system.

1.2 Exakt-micro-grinding system (Fig. 1):

The apparatus consists of a main unit with a rotating grinding plate which is either diamond coated and/or covered with sandpaper. The velocity of the grinding plate can be regulated. On top of the main unit, a block is attached parallel to the grinding plate. The block moves linearly stimulated by a winding spindle. A blank slide or specimen to be ground can be attached to the block by vacuum. The block also serves as a rinse and cooling system. The pressure in which the block will come in contact with the grinding plate is determined by individual weights. The linear velocity can be regulated. A micrometer screw is attached on the block which manipulates the thickness of the specimen to be ground. The microscrew is more or less the end point because, as soon as the determined thickness is reached the automatic grinding mechanism is stopped.

Mechanical Operation

The thickness of the object to be ground is regulated by adjusting the micrometer screw system attached to the microsystem. First, however, the zero point of

each specific grinding specimen needs to be determined using the following described procedure.

Zero Point Adjustment (Fig. 2):

The appropriate sandpaper and specimen are placed on the micro-grinding system. While the motors are running (grinding and parallel system) the micrometer screw is moved towards the contacts (underneath the system) until they are barely touching. This touching point will cause the motors to stop and is considered the determined zero point for the chosen (individual) specimen to register the thickness to be achieved. The micrometer screw is then moved in the opposite direction until the appropriate numbers appear on the indicator. Now, the micro-grinding system is ready. As soon as the specimen reaches the programmed thickness the grinding process is stopped automatically. It has been proven by experience that it is better to use sandpapers of different grits to grind the specimen gradually. Everytime, however, the sandpaper is changed, the programming of the zero point and the thickness adjustment of the specimen is mandatory.

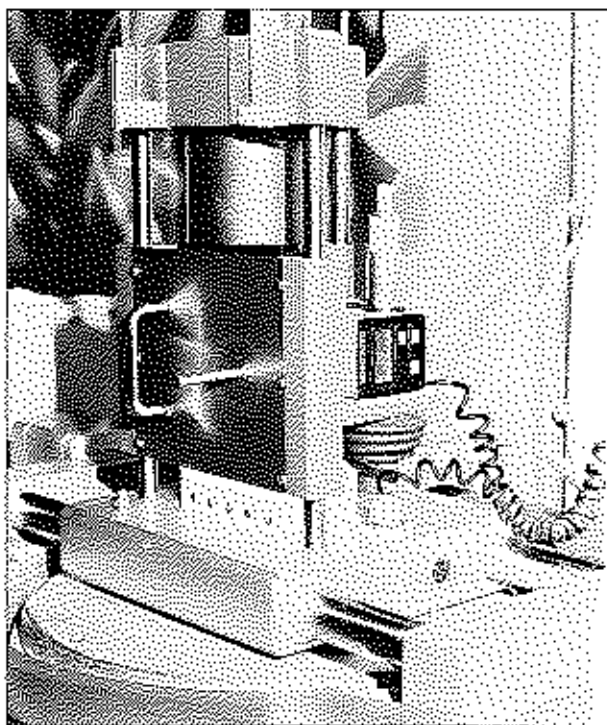


Fig. 2: Detailed view of Exakt-micro-grinding system, with attached vacuum holder (block) and micrometer screw system on the parallel guiding device.

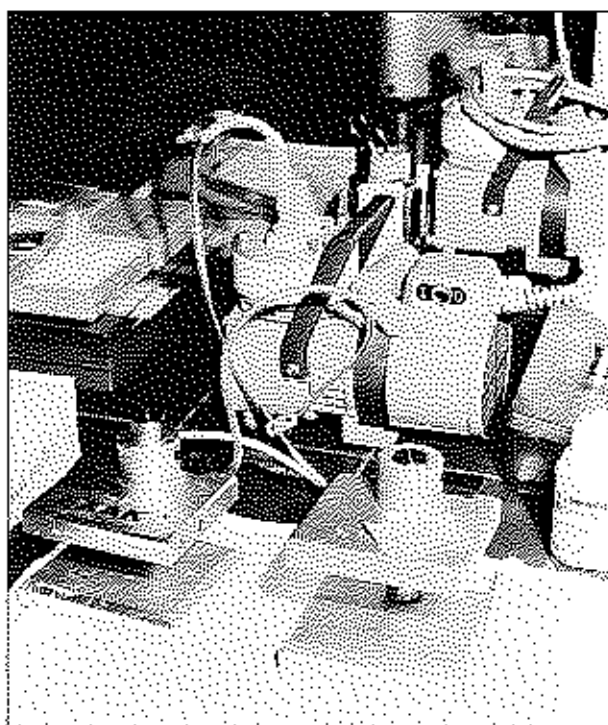


Fig. 3: Exakt-vacuum adhesive system, with vacuum pumps seen in the background (system is used to mount a plastic specimen block on a slide)

DESCRIPTION OF THE EQUIPMENT

2.1 Exakt-vacuum-adhesive system to mount the specimen block parallel to the slide (Fig. 3):

The adhesive system has a lower and upper plate. Both are connected by an axle mounted on the bottom plate which controls the parallel movement. A vacuum is attached underneath the upper coverplate to hold the slide.

Operation Procedure

The block is to be placed on the lower plate in such a way that the side to be examined is directed toward the lower plate. Underneath the upper plate a slide will be attached with the previous mentioned procedure, the Technovit 4000 used to mount the block on to the slide, is distributed evenly. At the end of the polymerisation stage, the vacuum will be released. Now the block and slide are connected in a parallel fashion; the slide is ready to be removed from the upper plate. The specimen, prepared as described above, is to be placed to the vacuum apparatus of the Exakt-micro-grinding system. The surface of the area of interest will be ground until paralleling is achieved and the specimen is at the face of the block.

2.2. Exakt-precision-adhesive press (Fig. 4)

The mechanical system is similar to the one described in 2.1. The upper plate however is transparent. (Fig. 4) The "final slide" is to be attached to the transparent plate by vacuum. The plate also allows easier control for bubble free gluing. Over the upper plate is a light source which serves the purpose of "Photopolymerization".

Operation Procedure

The parallel slide (final slide) with known thickness is lightened to the upper plate by vacuum. The slide with the parallel block is to be placed on the moveable base plate. The thickness is to be recorded. The slide of interest will be coated with precision photo-gluc. The base plate with the specimen will be pressed with uniform pressure by using weights against the upper plate. To insure bubble free mounting the glue should be distributed uniformly within 2-3 minutes. The polymerization process is initiated by a light source above the upper plate. The polymerization process is completed within 8-10 minutes.

2.4 Micrometer with digital display

Precision measurements are essential to obtain accurate results. A micrometer with digital display, Mitutoyo 2093 series digimatic micrometer is provided for this purpose.

Three specific measurements are required to determine the final thickness of the specimen, thin section.

1. thickness of specimen block + slide = A
2. thickness of parallel slide = B
3. thickness of total after gluing $A + B + X = C$

Thickness of glue (X) will be determined by subtracting A and B from C.

$$X = C - (A + B)$$

(Attention: All elements to be measured should have the same temperature - due to heat expansion).

2.5 Feeler gauge from 0,05 to 0,50 mm

Controls waste during separation procedure.

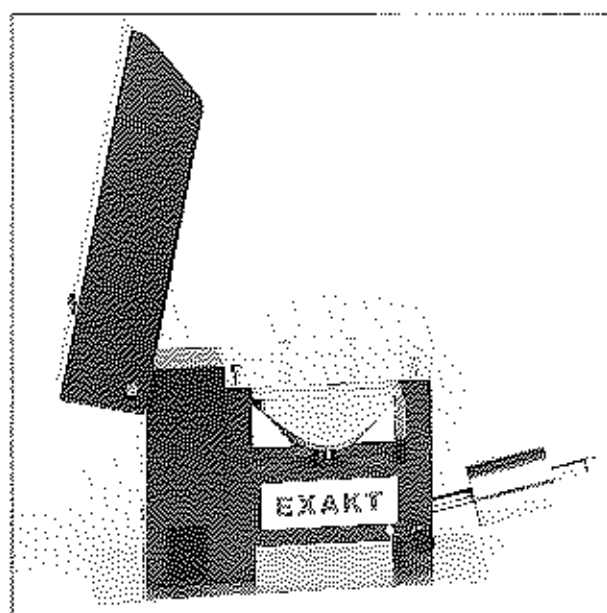


Fig. 4: Exakt-precision adhesive system for light activated bonding under direct vision.



Fig. 5 a

Fig. 5: Jaw segments including teeth, prepared by different methods for histological examination.
a) decalcification of the jaw-segment with loss of the dental enamel, celloidine, embedding and Haematoxylin-Eosin-stain.
b) plastic embedding without pretreatment prepared by applying the thin-section-system, retaining the dental enamel and calculus, toluidine blue stain.

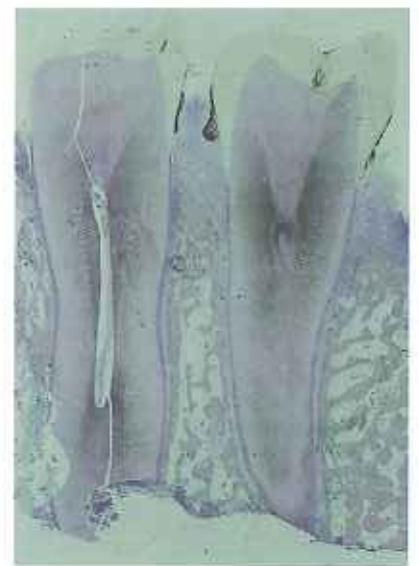


Fig. 5 b



Fig. 6: Tooth-bearing lower jaw-segment, of the mixed dentition. 2 mm thick with opening of pulp chambers and the bone marrow spaces to obtain better fixation, dehydration on plastic infiltration.



Fig. 7: Tooth bearing upper jaw segment of 2 mm thickness. Careful cutting with the cutting-grinding system, retaining the amalgam in the first molar and mucous membrane of the antrum.



Fig. 8: Slide of 2 mm thickness of upper jaw segment with splinted crowns and advanced carries at the crown margin.



Fig. 9: Slide of 2 mm thickness of an anterior maxillary segment, root canal with two gutta percha points in the right central incisor.



Fig. 10: Cross section through the shank of a hip endoprosthesis cemented in the femur.

2.6 Straight edge

The straight edge is to be used to detect unevenness of larger areas; i.e. to eliminate facets or concavities which occur, especially on transition areas of metal to bone or to soft tissues, while grinding the specimen with sandpaper.

3. Preparation of the tissues from the fixation

Tissue section of 2 to 4 mm thickness can be fixed well usually with good preservation. It is necessary to cut the tissue in smaller slices, when a histological examination of the pulp of a tooth or the bone marrow of the lower jaw is requested. (Fig. 7-II). Tissue samples from animal experiments present no difficulties, because a perfusion fixation can be performed.

Any tissue which can only be treated by immersion fixation may not be thicker than 2–4 mm. When preparing bones, which are surrounded with soft tissue, a pre-fixation with neutral formalin for 15 to 30 min. at 4° C is necessary. After pretreatment with formalin, the hard and soft tissues can be sliced in to 2 to 4 mm thick parallel slices without any problems by using the Exakt-cutting-grinding system. The maximum size of a specimen is 100 x 50 mm. The jaw segments can be held in either a longitudinal or transverse direction. It is important that the jaw be placed firmly against the moveable cutting table. The cutting table is moved toward the sawband by variable weights. Using this process, it is possible to cut a jaw with teeth and surrounding soft tissue into small slices (2 mm thick).

After a fixation period of 6 to 12 hrs., the parallel thin tissue specimen can be further processed by decalcification or the cutting-grinding technique.

4. Dehydration

The dehydration can be accomplished by either using increasing alcohol or glycolmethacrylate concentrations. The time of dehydration in the individual concentration depends on the thickness of tissue parts. With the manual dehydration procedure, 24–32 hrs. are recommended for each step. The dehydration time can be shortened by using an automatic dehydration system with agitation and vacuum.

5. Plastic infiltration

First step of the plastic infiltration is to use a mixture of pure glycolmethacrylate and an embedding medium in a ratio 1:1. The time of infiltration is about 8 hrs. when using 2–3 mm thick tissue specimen. The length of time from dehydration to final embedding can be shortened to 8–12 hrs, when using an automatic embedding apparatus with agitation and a vacuum system.

6. Embedding and Polymerization

The plastic infiltrated tissue slices are placed in embedding molds. A so called plastic fixation medium can be used to prevent the soft tissue from lifting away from the bottom plate. The final polymerization process is accomplished by a light with 450 nm wave length. The polymerization takes place in two or three steps.

Step 1: Using low intensity light and maintaining temperatures below 40 degrees Centigrade, the embedding media is extensively polymerized. Approximate time for this step is 4 hours.

Step 2: Using a higher intensity blue light embedding media which has been infiltrated into tissue is completely polymerized. For specimens 5 mm thick or less the approximate time for this step is 4 hours. For specimen 5–16 mm in thickness the polymerization time should be extended up to 10 hours depending on the actual thickness of the specimen and the type of tissue.

Step 3: Supplemental polymerization using Benzoyl Peroxide*. In the event thicker specimens (those thicker than 5 mm) contain predominantly soft tissue or high concentrations of blood polymerization can be enhanced by adding Benzoyl Peroxide* to the Kulzer Technovit 7.200 VLC during the final infiltration step. After proceeding with the first two steps of polymerization with the Exakt-light-polymerization unit the specimen can be placed in an incubator at 50 degrees Centigrade for up to 12 hours to complete polymerization.

* If Benzoyl Peroxide is used, the special safety rules of the country are to be attended.

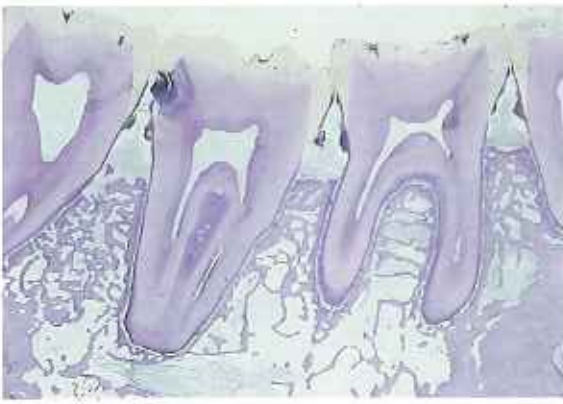


Fig. 11: Thin section of a lower jaw of a 32-year-old man. Concretions and plaque within the interproximal spaces, distal of the left second molar.



Fig. 12: Thin section of the lower jaw of a 28-year-old female. Ceramic veneered, precious metal crowns on the left first and second molars.



Fig. 13: Section of a tooth with dentin/tartrate/cementum and attached epithelium. On the surface of the dentin an ectopic enamel pearl. Slight subepithelial inflammation in infiltrate. Thin section, Toluidine blue stain.

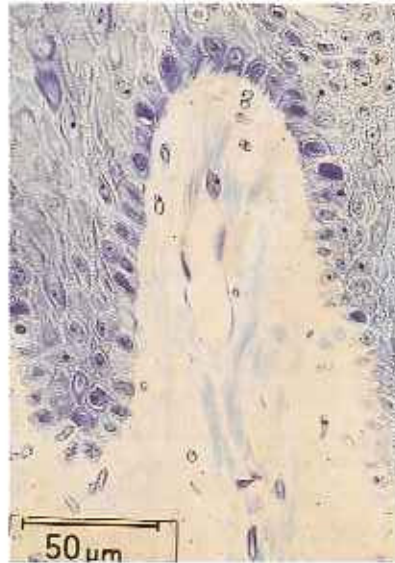


Fig. 14: Section of the free gingiva, non-inflamed connective tissue. (Thin section, Toluidine blue stain.)



Fig. 15: Bridge abutment (left illustration) extended too far subgingivally. (Thin section, Toluidine blue stain.)



Fig. 16: Interdental area with alveolar bone (limbus alveolaris) interdental papilla and parts of the fiber apparatus.

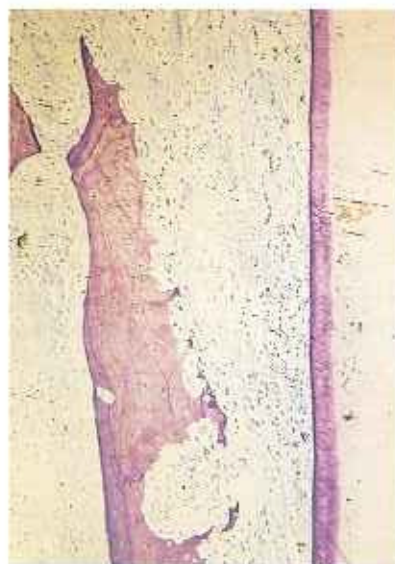


Fig. 17: Vertical bone loss in the socket. Right side of picture-section of the tooth root. (Thin section, Toluidine blue stain.)



Fig. 18: Area enlargement of Fig. 17; poly-nuclear giant cells within bone socket. (Thin section, Toluidine blue stain.)

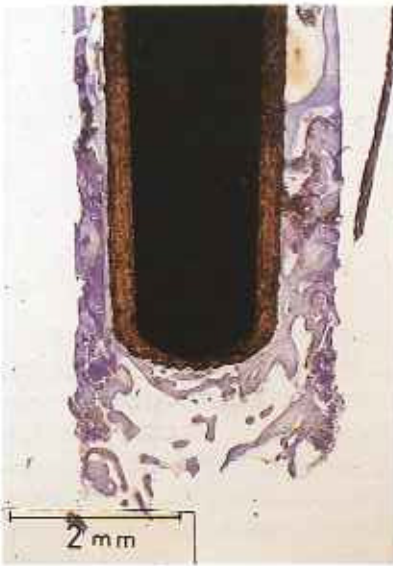


Fig. 19: Ceramic coated titanium implant in direct contact with the bone. (Thin section Toluidine blue stain.)



Fig. 20: Part of Fig. 19, direct contact of ceramic and bone. (Thin section. Toluidine blue stain.)

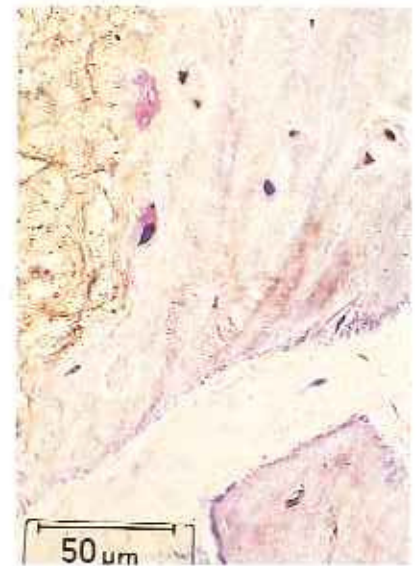


Fig. 21: Part of Fig. 20, ceramic-bone contact zone. (Thin section. Toluidine blue stain.)



Fig. 22: IMZ-Implant with deep pocket formation and direct bone contact in the lower third. (Thin section. Toluidine blue stain.)



Fig. 23: Part of Fig. 22, interface of metal and bone with zone of corrosion interspersed. (Thin section. Toluidine blue stain.)



Fig. 24: Peri-implanted bone with infiltration of corrosion products in the cytoplasm of the osteocyte and within the area of mineralization. Thin section. Masson-Goldner stain.



Fig. 25: Hydroxylapatite ceramic in direct contact with bone. (Thin section. Toluidine blue stain.)



Fig. 26: Same as Fig. 25, but in polarized light.



Fig. 27: Hydroxylapatite ceramic in direct contact of the bone (at the right). (Thin section. Toluidine blue stain.)

7. Preparation of the block to obtain a parallel surface

The polymerized tissue block is removed from the embedding mold. The next step is the preliminary grinding which brings the area to be examined closer to the surface. In order to obtain a flat surface, the opposite side of the plastic block is mounted on a slide by using Technovit 4000. Technovit 4000 causes no damage to the slide. The vacuum-adhesive apparatus is used for the mounting procedure. The slide is held by vacuum in the upper part of the press. Auto-polymerizing resin is placed on the back side of the specimen block. Then, the side of the specimen to be examined is placed against the lower plate of the apparatus. Next the upper plate with the attached slide is lowered carefully, until an adequate contact occurs, the plate then will be secured with a screw. As soon as polymerization is finished the block is ready to be polished with the micro-grinding system.

8. Preparation of the surface of interest

The block which is mounted on the slide is placed into the vacuum apparatus attached to the micro parallel grinding system. The grinding table is covered with 1200 grit sandpaper. The grinding process is completed when all the tissue segments to be studied are exposed to the surface. Simultaneously, the surface of the block must be parallel with the slide. Unevenness within the surface of the specimen can be detected with a straight edge. Differences of 3 to 5 μm are acceptable, when large specimens of about 90–100 mm diameter are used. After the definitive surface of the specimen to be studied is reached it is finished with 4000 grit sandpaper to create as smooth surface as possible. 2–4 reference points must be recorded.

9. Affixation of a parallel slide onto the block.

The parallelism of the slide must be verified. If discrepancies are present, the slide needs to be adjusted with the Exakt-micro-grinding system. When parallelism is obtained, treatment with sandpaper of 4000 grit is used to smooth the surface of the slide. The final thickness of the slide is to be recorded in a protocol (2 measurements). Before the precision affixing, the surface of the block and slide is to be cleaned

with an organic solution (petroleum or benzine). Don't use any acetone at all. The cleaned slide is to be placed with the side to be mounted upside down into the precision press. Vacuum is used to hold the slide. The precision adhesive is delivered (thin) onto the prepared block surface. The specimen is put on the lower plate. The lower plate is pressed toward the upper plate after loosening the safety device. The same pressure is maintained by a variable weight. If bubbles occur the slide and block can be separated. After cleaning the sample and slide by benzine the same process can be repeated. When perfect mounting takes place, a uniform distribution of the precision adhesive in "glue space" will occur within 2–5 min. If the adhesion is satisfactory, photopolymerization is initiated by applying the curing light. The polymerization is completed within 15 to 20 min. The excess glue, which flows out from the glue space, remains soft because of the influence of the air (oxygen) and therefore, can be removed easily with a towel. Before the separation cut is started, the glue thickness is to be calculated as described in 2.4.

$$X = C - (A + B)$$

10. Preparation of the separation cut by using the cutting-grinding system

It is possible to place either slide onto the vacuum apparatus attached to the cutting-grinding system. Because the photopolymerized adhesive area remains visible, it is advisable, however, to select the parallel slide. It is helpful to use a paper sticker (100 μm) on the edge of the slide to adjust the chosen thickness. While the sawband is slowly moving, the slide is to be moved toward the parallel guidance, by using the micrometer screw, until the paper sticker barely touches the blade without being damaged. The thickness to be expected is about 100 μm , including the thickness of the glue. Advancing weights of 50–100 grams are sufficient during the cutting process. Because the finished side of the slide is placed away from the vacuum system, the cutting process can be monitored. Any interruptions or changes of the weights while the cutting procedure is in progress will cause unevenness on the two surfaces that are produced.

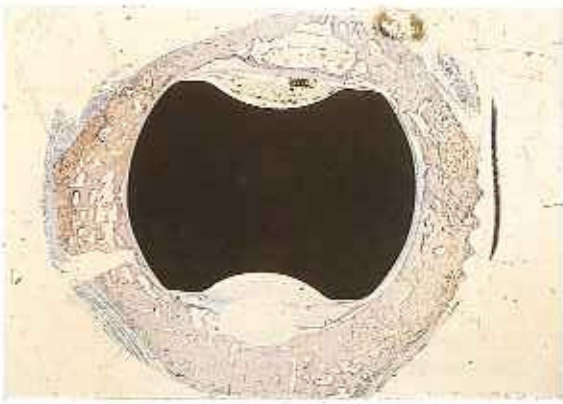


Fig. 28: Thin section of a cemented shank in the femur of a hip endoprosthesis. (compare Fig. 10, Toluidine blue stain).

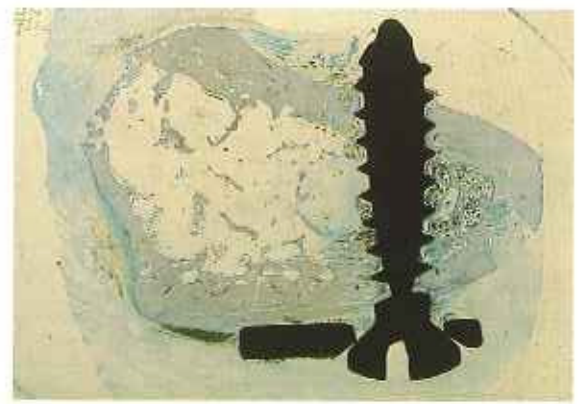


Fig. 29: Thin mandibular cross section with a screw and plate in osteosynthesis. (Thin section, Toluidine blue stain.)

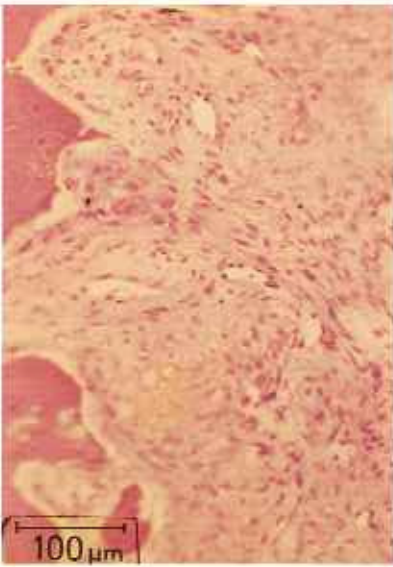


Fig. 30: Alveolar bone with bone apposition (osteoid and osteoblasts) and bone destruction by osteoclasts. Thin section, Haematoxylin-Eosin stain.

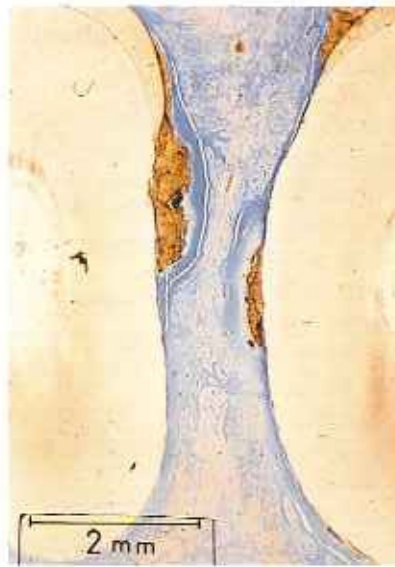


Fig. 31: Interdental region with horizontal section of teeth. The calculus on the teeth extends subgingivally. Thin section, Giemsa stain.

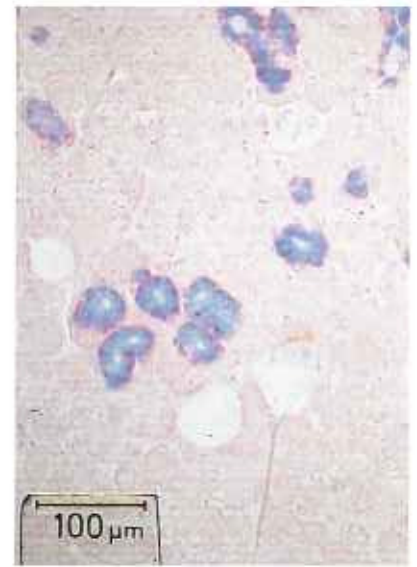


Fig. 32: Submandibular gland is with illustration of the mucous acini. Thin section, Astra-blue stain.



Fig. 33: Alveolar bone with section of tooth. Osteoid margins (red) on the surfaces of the bone trabeculae. Thin section, Masson-Goldner.

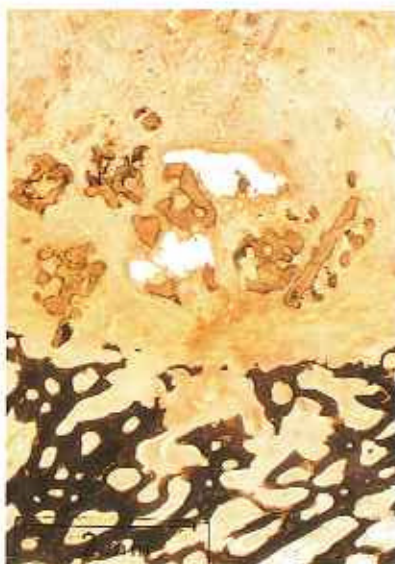


Fig. 34: Lower jaw with defective filling through porous hydroxylapatite ceramic. Thin section, Kossa-stain.



Fig. 35: Edentulous mandible (red) and overlapping alveolar mucosa. Thin section, Van Gieson stain.

11. Preparation of the final thin section

The thickness of the section is calculated by subtracting the thickness of the parallel slide including the thickness of the glue, from the thickness of the total cut specimen. For example, if the total thickness of the grinding specimen is 1655 μm , the parallel slide 1550 μm , glue thickness 5 μm – the separated section is 100 μm thick. The calculated value of 100 μm is considered as the reference point for the micro-grinding system. Before starting the grinding procedure, the zero point is to be determined. In order to do that, the slide with the specimen is placed on the vacuum block. The grinding table is covered with sandpaper. The specimen is carefully lowered by using the micrometer screw until the electric current for grinding table and parallel movement is interrupted. A control light will indicate that the zero point is established. This procedure is performed while the motors are running. Then the actual amount to be ground away is programmed into the micrometer screw system. The apparatus will be stopped automatically after the programmed thickness is removed. The last polishing of the surface is accomplished by using sandpaper with a fine grit. Minor grinding marks are eliminated with the coverslip medium, after the staining procedure. Metals in connection with bones and soft tissues must be ground with a diamond coated plate, because sandpaper will not provide an absolutely even surface. This phenomenon is explained by the fact that metal presses into the sandpaper, therefore, the surrounding soft and bone tissue will be ground off faster.

12. Staining of thin sections

We are routinely using for the daily diagnostic histology section, Toluidine blue because the staining procedure is quick and simple. The metachromatic stain allows conclusions about bone appositions and bone resorptions. (Fig. 11–18, 19–23, 25, 27–29).

Toluidine blue-staining:

1. agitate in 10% H_2O_2 , 5 minutes
2. rinse in water
3. wipe dry
4. staining in Toluidine blue solution, 20 minutes
5. rinse in water
6. Wipe dry carefully. If the plexiglas-slide is blue it can be wiped with 70% alcohol. Not the specimen!
7. After 5 min. drying or waiting time over night coverslipping with Technovit 7200 VLC.

8. Polymerization on top of the plastic plate of the EXAKT Precision-Adhesive-Press.

Ingredients:

Sodium Tetraborat (Borax)
Toluidine blue (Chroma)
Pyronin-G (Merck 7517)

Application: embedded tissue in plastic, pre-treated with H_2O_2 .

Preparation of 1 liter staining solution

Solution A:	Solution B:
800 ml distilled water	200 ml distilled water
8 g Sodium Tetraborat	2 g Pyronin G
8 g Toluidine blue	Mix 15 min. with a
Mix 15 min. with a	magnetic stirrer.
magnetic stirrer.	

Solution A and B are to be mixed with a magnetic stirrer for 15 min.

Then mixture is to be filtered 2 x.

Of course other tissue-staining methods are possible, when using Technovit 7.200 VLC.

Haematoxylin-Eosin (Fig. 30):

1. clean surface of the slide with acetone-alcohol 1:1
2. stain 40 min. in Haemalaun (Shandon)
3. rinse 1 min. in glacial acetic acid
(1 ml in 100 ml dist. water)
4. water 10 min. in running tap water; to dye blue
5. stain 5 min. in Eosin
(Eosin-y-water-soluble, Shandon)
6. rinse with running water
7. differentiate briefly in 80 % alcohol
8. dip the slide quickly in 96 % alcohol
9. dip quickly once in xylene
10. coverslip

PAS reaction:

1. 10 min. 0.5 % periodic acid solution
2. rinse slide in distilled water, 2 jars
3. place slide for 30 min. in Schiff's reagent.
4. place slide 3 x for 2 min. in wash solution
5. rinse slide for 5 min. in running tap water
6. stain 30 min. in Haemalaun
(Mayer in incubator 60° C)
7. dye blue 10 min. in running tap water
8. dry slide and coverslip

Production of wash solution:

30 ml sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$) and 30 ml of 1 N hydrochloric acid in 600 ml distilled water

Ingredients of the Schiff's reagent

Pararosanilin (Sigma, P 1528, Merck)

Hydrochloric acid (1 N)

Sodium metabisulfite

Active charcoal

distilled water

Production of Schiff's reagent:

Dissolve 10 g Pararosanilin in 3L flask by adding 2000 ml distilled water cool down to 50°C.

Add 200 ml 1 N HCl and cool down to 25° C.

Dissolve in this solution, 10 g ($\text{Na}_2\text{S}_2\text{O}_5$)

Place for 24 hrs in dark environment. Then add 10 g active charcoal, mix well.

After filtration store in a brown bottle.

Astra Blue stain (Fig. 32):

1. 3 min. in 3 % acetic acid
2. stain 30 min. in Astra Blue solution
3. rinse with tap water
4. stain for 30 min. in Kernechtrot
5. rinse with tap water
6. dry slide and coverslip

Ingredients:

Astra Blue (1278 Merck)

Kernechtrot (15939 Merck)

Production of Solution:

1. Astra Blue solution:
dissolve 2 g Astra Blue in 100 ml 3 % acetic acid, overnight.
2. Kernechtrot (nuclear fast red stain):
1 g Kernechtrot
dissolve 50 g aluminum sulfate in 1000 ml hot distilled water;
filter after cooling down.

Masson-Trichrom-Goldner (Fig. 24, 33):

1. stain 15 min. Weigerts ferric Haematoxylin
2. rinse 5 min. in running tap water
3. stain 7 min. in Masson-solution (Goldner I)
4. rinse with 2 % acetic acid
5. stain 5 min. phosphormolybdaenum-acid-Orange-G
6. rinse with 2 % acetic acid
7. stain 15 min. in "light green", 60° C incubator
8. rinse with 2 % acetic acid
9. dip slide briefly in distilled water, to wash off the acetic acid
10. dry slide and coverslip

To consider: Prepare Weigerts ferric Haematoxylin shortly before use. Use equal parts of 1 and 2 solutions.

Ingredients:

Acid fuchsin (Rubin S, Merck)

Xylidin-Ponceau (1 B 207, Chroma)

Azophloxin (1 B 103, Chroma)

Light green (yellowish, Merck)

Production of the solutions:

A. Masson-Solution (Goldner I)

1. dissolve 1 g acid fuchsin in 100 ml distilled water and 1 ml glacial acetic acid
2. dissolve 1 g Xylidin-Ponceau in 100 ml distilled water and 1 ml glacial acetic acid
3. dissolve 0.5 g Azophloxin in 100 ml distilled water and 0.2 ml glacial acetic acid.

Mix 33 ml of the acid fuchsin in solution and 66 ml Xylidin-Ponceau-solution.

Mix 100 ml of this solution with 20 ml Azophloxin solution and 880 ml 0.2 % acetic acid.

The finished mixture is identical with the Masson-Goldner-Solution (Goldner I).

B. Orange-G

1. dissolve 10 g Orange-G in 500 ml distilled water
2. dissolve 15 g Molybdato-phosphor acid (Merck) in above (1) solution.

C. Light green

1. mix 0.5 g light green and 1 ml of glacial acetic acid into 500 ml distilled water.

Silver stain of Gomori:

1. 3 min. 0.25 % potassium permanganate solution
2. rinse with distilled water
3. bleach in 3 % potassium metabisulfite-solution
4. water 5 min. in tap water
5. sensitize 1 min. in 2 % ferric ammonia alau-solution
6. water 2 min. in tap water
7. rinse with distilled water, 2 jars
8. impregnate 1 min. in silver solution
9. dip in distilled water (5–10 sec.)
10. reduce 5 min. in 4 % neutral formalin
11. rinse with distilled water, 2 jars each 3 min.
12. gild 5 min. 0.1 % aurous chloride
13. rinse with distilled water
14. reduce 1 min. in 3 % potassium meta-bisulfite-solution
15. rinse with distilled water
16. fix 2 min. in 5 % sodiumthiosulfate-solution
17. water 5 min. in tap water
18. stain 40 min. Kernechtrot
19. rinse with distilled water
20. dry slide and coverslip

Production of silver solution:

10 ml of a 20 % solution of silver nitrate, a brown precipitation develops. Add drop by drop liq. ammoni-caustici, until the precipitation just disappears. Fill up with distilled water to the four fold.

Ingredients:

Kernechtrot (15939 Merck)

Gold chloride = sodium tetrachlor-aureat (III)
(60550 Fluka AG Switzerland)

Movat (silver impregnation):

1. 20 min. 0.5 % periodic acid
2. water in distilled water, 2 jars each 5 min.
3. stain for 1¹/₂ hrs. in silver solution, 60° C in incubator
4. rinse with distilled water, 2 jars
5. 5 min. 3 % sodium thiosulfate
6. water 5 min. tap water
7. dry slide and coverslip

Production of silver solution:

Add to 40 ml of a 3 % hexamethylen-tetramin-solution 5 ml of a 5 % silver nitrate-solution, mix well. Add 5 ml of a 2 % borate solution; after 5 min. filter 2 x.

Giemsa (Fig. 31)

1. stain 15 to 30 min. in giemsa-solution, 60° C incubator
2. rinse with distilled water
3. dip a few seconds in a cuvette containing glacial acid water (8 drops glacial acid in 100 ml distilled water)
4. 3 cuvettes containing 96 % Isopropylalcohol (keep in last cuvette for 5 min.)
5. 3 x for 2 min. isopropanol
6. dip briefly in xylene
7. coverslip slide

Substrat: Giemsa-Solution (9204, Merck)

Van Gieson-connective tissue stain (Fig. 35)

1. clean slide with acetone-alcohol (1:1)
2. stain 15 min. in Weigert's ferric Haematoxylin
3. water 5 min. in running tap water
4. stain 4 min. in van Gieson-mixture (picrofuchsin)
5. rinse with water
6. differentiate briefly in 80 % alcohol
7. dip once in 96 % alcohol
8. dip once in xylene
9. coverslip

Notice: set up Weigerts ferric Haematoxylin just before to be used (Solution 1 and 2 equal amount)

Production of the van Gieson-mixture: 100 ml filtered saturated, aqueous picric acid + 10 ml 1 % acid fuchsin solution, acid fuchsin (1 B 525, Chroma)

Combined Elastin-van Gieson-stain:

1. clean slide with acetone-alcohol (1:1)
2. stain 30 min. in Resorcin-Fuchsin, according to Weigert.
3. rinse with tap water
4. differentiate briefly in 0.5 % HCL-alcohol
5. water 10 min. in running tap water
6. 7 min. nuclear staining according to Weigerts ferric Haematoxylin
7. water 10 min. in running tap water
8. stain 4 min. in van Gieson-mixture
9. rinse with tap water
10. differentiate shortly in 80 % alcohol
11. dip once in 96 % alcohol
12. dip once in xylene
13. coverslip

Reagents: Resorcin-Fuchsin (11430, Chroma)
Van Gieson-mixture (see recipe van Gieson)

Resorcin-Fuchsin after Weigert:

Dissolve 1 g Resorcin-Fuchsin in 4 ml 25 % nitrite acid and fill up to 560 ml with 70 % alcohol.

Azan of Heidenhain:

1. 5 min. Aniline-alcohol (1 ml Aniline-oeslin 1000 ml distilled water)
2. dip briefly in 80 % alcohol
3. rinse with distilled water
4. 30 min. nuclear staining with azocarmine, incubator 60° C
5. rinse with distilled water
6. differentiate in Aniline-alcohol until the nuclei appear satisfactory while decolorizing the rest of the tissue, adding few drops of water the process is quickened
7. wash quick in acetic acid alcohol (1 ml glacial acetic acid in 100 ml 96 % alcohol) to remove the Aniline
8. 30 min. 5 % phosphor-tungstenic-anhydride
9. rinse with distilled water
10. stain 30 min. in Aniline-goldorange
11. rinse with distilled water
12. dry slide and coverslip

Production of solutions:

Azocarmine: dissolve 0.1 g azocarmine G in 100 ml distilled water. Boil up briefly. Filter after cooling down 100 ml of the filtered solution is to be mixed with 1 ml glacial acetic acid.

Aniline-gold-orange: dissolve 0.5 g water soluble Aniline Blue and 2 g Orange G in 100 ml distilled water. Add 8 ml glacial acetic acid boil and filter after cooling down. This stock solution is to be diluted 3 x with distilled water before used.

Rhodamin B-stain to determine keratin:

1. agitate the slide 2 min. in 30 % H₂O₂
2. rinse with distilled water
3. stain 10 min. in Toluidine blue solution
4. rinse good with distilled water
5. stain 10 min. in 0.1 % Rhodamin-solution
6. rinse briefly with distilled water
7. dry slide and coverslip

Production of Rhodamin B-solution:

Stock solution: dissolve 0.1 g Rhodamin B (Merck) in 100 ml buffer solution

acetic acid-sodium, acetate buffer pH 3,6:

185 ml 0,1 N acetic acid

15 ml 0,1 molar sodium acetate pH 3,6:

Toluidine-solution (see Toluidineblue-stain recipe)

Fibrin stain of Ladewig:

1. clean slide with acetone-alcohol (1:1)
2. stain 10 min. in Weigert's ferric Haematoxylin
3. water 5 min. in running tap water
4. stain 5 min. in Aniline blue-acid fuchsin-gold-orange-mixture (Ladewig-solution)
5. rinse in distilled water
6. dry slide coverslip

Production of Ladewig-solution:

Dissolve 1 g Aniline blue water soluble (Chroma) and 4 g Orange-G (1 B 221, Chroma) in 200 ml distilled water. Add 16 ml acetic fuchsin (Rubin S, Merck) boil briefly and filter after cooling down.

Notice: set up Weigerts ferric Hematoxylin shortly before use. Solution 1 and 2 are combined in equal parts.

Kossa-stain (Fig. 34)

1. place slide for 1-1^{1/2} hrs, in 5 % silver nitrate solution exposed to daylight, to put it in directly in sunlight is best. The length of the procedure can be shortened or prolonged depending on color intensity. The tissue should show a dark brown almost black color.
2. rinse several times with distilled water
3. 5 min. in 5 % sodiumthiosulfate-solution
4. water 10 min. in tap water
5. stain 30 min. in Kernechtrot (37° in incubator)
6. rinse with distilled water
7. dry slide and coverslip

Berliner-blue-reaction:

1. place slide for 1/2 hr. in a mixture of potassium-ferrocyanide and 1 % hydrochloric acid, 60° C incubator
2. rinse with distilled water
3. stain 1 hr. in Kernechtrot 60° C incubator
4. rinse with distilled water
5. dry slide and coverslip

Production of solution 1:

1 g potassium ferrocyanide in 50 ml distilled water add 50 ml hydrochloric acid, set-up solution shortly before to be used.

Kernechtrot see receipe "Astra Blue"

The staining method were performed on specimens with 10-5 µm thickness, obtaining good results. Thicker samples show differences in staining results, because the coloring power demonstrates differences in its infiltration retention.

13. Coverslipping of stained specimens:

All media are to be used as in the paraffin histology.

Best results are by Technovit 7,200 VLC. Polymerization at the Exakt-precision-adhesive press takes 15-20 min.

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