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Visualization of the symmetry of regional zones of dentin of a human molar tooth in polarized light

© *V.M. Zolotarev*[1,2]

[1] ITMO University,
197101 St. Petersburg, Russia
[2] Federal State Budget Enterprise Consulting and Diagnostics Center with Polyclinic,
197110 St. Petersburg, Russia
e-mail: vm-zolotarev@mail.ru

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In the region of the regional zones of the dentin crown of a human molar tooth, the symmetry of the dentin microstructure was studied using the visual optical polarization method. It has been shown that dentinal tubules in the vicinity of the molar cusps are organized in the form of bundles, which often have axial symmetry. The symmetry axes of the bundles coincide with the corresponding pulp horns and are oriented towards the center of the tubercles of the tooth crown. Within the growth zones that form the tubercles, peculiar optical effects similar to conoscopic figures in the form of a „Maltese cross"were visually detected. The nature of such figures indicates the ordered structure of growth zones formed from optically anisotropic tubes around a single center. The data obtained by the optical method correlate well and informationally complement diffusion methods based on staining of dentinal tubules. It has been shown that the visual polarization optical method can be used to study different types of organization of the microstructure of dentinal tubules.

Keywords: symmetry, crossed polarizers, optical anisotropy, dentinal tubules, growth zones.

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Introduction

The recent discoveries related to the mechanism of cascade regulation of permanent teeth development form a scientific base for understanding the ways and mechanisms for evolution of the shape and size of teeth in all mammals. The process of tooth growth both at genetic and at functional (food type impact) level is determined by the ratio of the activator and the inhibitor strictly set in ontogenesis, with their interaction that controls the stages of formation and differentiation of epithelial and mesenchymal growth cells [1]. Therefore, the evolution of the mammal species is determined within the limits of the present range of variants permitted by ontogenesis. These theoretical scientific discoveries have recently been confirmed in practice, having demonstrated the possibility of replacing a lost tooth with its analogue grown in vitro in principle [2]. As a result of these discoveries, the possibilities are being discussed more actively for the development of bioengineered constructs — teeth with the application of bioengineered cell matrices (scaffolds), which are introduced to regenerate the bones [3].

The therapy of full teeth replacement is deemed to be an attractive concept for regenerative therapy of the next generation as a form of a bioengineered replacement of organs. For regeneration of the entire tooth, a new method of 3D manipulations with cells was developed, which was named „the organ germ method" [2]. This method simulates the multicellular conditions of assembly and epithelial-mesenchymal interactions in organogenesis. A bioengineered tooth germ creates a structurally correct tooth *in vitro*, which successfully erupts and develops when transplanted in the oral cavity. Such a bioengineered unit (a structural block) comprises a formed tooth, a periodontal ligament and alveolar bone, and this tooth unit was transplanted to the adult jaw bone by bone integration [4]. The bioengineered teeth could also perform the physiological functions of teeth, such as chewing, the periodontal ligament function and response to hazardous stimuli.

Currently it is expected that the regenerative therapy of teeth for recovery of the tooth tissues and replacement of the entire tooth will become a new therapeutic concept with complete recovery of the tooth physiological functions. At the same time the practice of forming a tooth with the preset properties requires preparatory research, which is necessary to manage the mechanisms of its dentogenesis. The important stages of studying the properties of an artificially grown tooth that characterize its quality would be the elements of symmetry and asymmetry of dental arches, as well as individual components in such an arch [5].

The studies of the morphological parts in the appearance of a molar crown show that the photos (patterns) below in the crossed polarizers are of heuristic nature, making it possible to analyze the issues of tooth development and evolution [1] and, in particular, to establish the ratio between the properties of the molar crown and its root [6]. Minor variations of the parts of the crown shape and the number of tooth roots are the signs that are most liable to the evolutionary changes. A tooth crown is also widely used as a tool to establish the principles of development that relate the genotype with the phenotype [7]. Therefore, the additional information about the properties and features of the tooth development may be provided by studying the features of the symmetry elements during tooth formation [5].

The symmetry is in general inherent in the Nature [7–9], and in live organisms it is related to their adaption to the environment, to their vital capacity [10–12]. The concept of symmetry and the role of tooth evolution in the literature are studied at the morphological level in respect to the composition of the molar crown shape [5]. There are different points of view regarding the development and formation of teeth types in process of tooth evolution from a simple conical shape [5–7]. Properties of dentinal tubules, on the basis of which a tooth is formed, were studied in detail by various physical methods [11–17], however, the features of the mutual arrangement of the dentinal tubules, which directly participate in the process of dentin growth and thus determine the shape of a certain tooth from the positions of symmetry have not yet been considered. The purpose of this paper — to demonstrate using the optical method the differences in the symmetry of growth centers provided for by the features of the structural arrangement of dentinal tubules that form a human molar tooth crown.

Physical properties and structure of dentin

The dentin structure has a complex multi-level hierarchical composition and is formed by micro- and nanostructures from peritubular dentin (PTD), from which the dentinal tubules are actually formed, and intertubular dentine (ITD), which fills the space between tubules. The peritubular dentin represents a relatively dense mineral tissue that surrounds the crown dentine tubules of the tooth. It mostly consists of apatite nanocrystals with a small amount of collagen. The arrangement of PTD nanocrystals is similar to the structure of the adjacent intertubular dentin. The latter comprises a relatively high quantity of collagen, and apatite nanocrystals are closely bound to the collagen matrix. It is deemed that the interface between PTD and ITD is homogeneous in structure [18]. Dentinal tubules have a S-shaped path from the pulp to the dentin-enamel junction (DEJ). Throughout their length to the periphery they may contact to each other via thin lateral branches. The dentin-enamel junction's dentin tubules end with a Y-shaped split [13–15]. The number of tubules per unit of area is around $54{,}000\,\mathrm{mm}^{-2}$ in the inner dentin, $30{,}000\,\mathrm{mm}^{-2}$ in the middle dentin and $8{,}000\,\mathrm{mm}^{-2}$ in the outer dentin. The diameter of tubules is around $1.9\,\mu$m in the inner dentin, $1.4\,\mu$m in the middle dentin and $1.2\,\mu$m in the outer dentin [19]. The cavities of multiple tubules contain growth cells in the form of odontoblastic processes responsible for dentin growth. These cells occupy a large part of the space inside tubules and therefore control the movement of transudate in the system of microopenings — transudate being the liquid in the tooth pulp. The tubules also contain nerve cells that are liable for response to the external stimuli. The odontoblastic process for $50-160\,\mu$m accompanies the bundle of nerve fibers in the concavities of the process surface [20,21]. The dentin sensitivity depends on nerve fibers of A-beta and A-delta types, their endings are located in the area of the tooth pulp-dentin. Several hypotheses of stimuli transmission via dentin have been proposed. They include direct stimulation of nerves, dentin receptors, hypotheses of ion diffusion and hydrodynamic hypothesis. The most common method of nerve activation related to dentin sensitivity is the hydrodynamic mechanism [22–24]. Abundant pores and cracks in PTD indicate that PTD does not determine the strength of the mechanical structure of dentin. The main role of PTD — transportation of the pulpal fluid via tubules to support the vital capacity of dentin as a human tissue [22].

The unity of the biological base of dentin that is specific for mammals made it possible to extend the model study of some tooth properties in *in vitro* and *in vivo* modes to animals and to compare those to the human tooth properties [25–27]. In particular, some papers note that by some parameters (shape, size of the crown) the human tooth molars may be compared to the permanent teeth of a pig (large size, thick enamel, crown shape). However, compared to the human teeth, the permanent teeth of pigs develop much faster. In virtue of the difference between the properties of the regional areas in a molar tooth the studies are often carried out specifically in the area of a certain tubercle on a large number of test objects [20,25–27]. Therefore, the teeth of domestic pigs are a convenient model biosystem for solving the practical objectives of odontology and to study the process of human tooth bioremineralization [28–31].

The features of the biological material — dentin, from which a tooth is formed, are related to the anisotropy of dentin, which is due to the properties of the tubules as such. Anisotropy of tubules and their structural arrangement result in anisotropy of a whole range of tooth properties: permeability and sensitivity [16,32], as well as optical [33–40], mechanical [41,42] and electric properties [43,44]. Dentin tubules may be compared to uniaxial crystals, which are similar to hydroxyapatite (GAP) in their properties. GAP crystals have a hexagonal type of the crystal system, are optically uniaxial, negative. The optical axis of GAP matches the crystallographic axis (c), which is aimed along the largest size — the longitudinal axis of the crystal.

The nanocrystals of GAP in the structure of the human peritubular dentin have the average length of 36.00 ± 1.87 nm, width of 25.57 ± 1.37 nm and thickness of 9.76 ± 0.69 nm. They consist of the plates with the average width to thickness ratio of 2.61, each representing a flattened hexagonal prism. The longitudinal axis of the GAP nanocrystal is aligned along the axis of the dentinal tubule. The order of individual GAP nanocrystals inside a tubule determines the optical properties of tubules in the form of

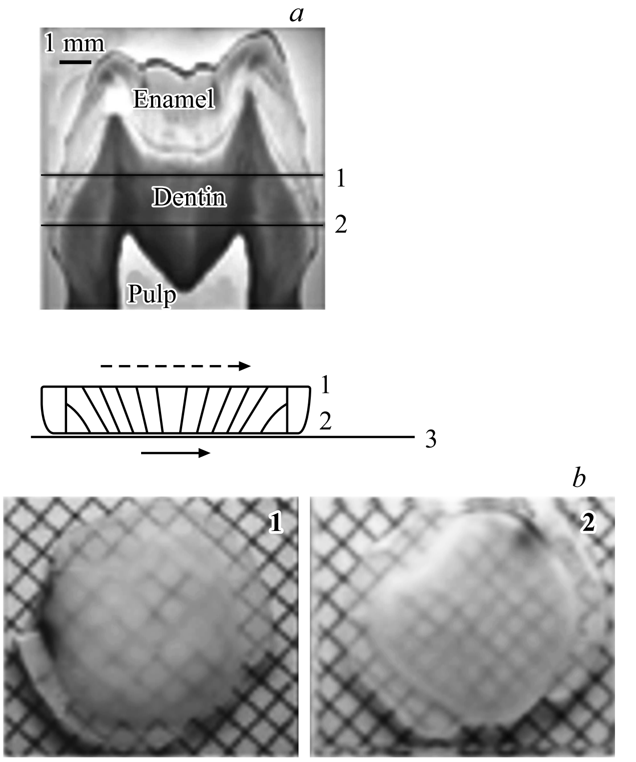

Figure 1. (*a*) The vertical cross section of the molar tooth, showing the planes 1–2 of the thin section (at the top), and the pattern showing the arrangement of dentinal tubules and contact of the plane 2 of the thin section with the test object 3 (at the bottom); the arrows indicate the change in the size of the visual image upon imposition of the plane 2 of the thin section onto the test object; the solid arrow indicates the size of the test object image without the thin section, the dashed arrow indicates the size of the test object image in the contact with the thin section. (*b*) Photo: 1 — plane of thin section 2 in contact with the test object, 2 — plane of thin section 1 in contact with the test object.

a combination of uniaxial nanocrystals. The optical axis of GAP is directed along the axis of tubules. The average value of the dentin refraction index is $n^1 = 1.50 \pm 0.02$ [45], the birefringence is $\Delta n = n_e - n_o = 0.005$. Natural crystals have minor dichroism, making them yellowish. Since the relative refraction index of tubules is $n = n_{\mathrm{ITD}}/n_{\mathrm{PTD}} = (1.45{-}1.5)/1.59 < 1$, when the ends of dentinal tubules are illuminated at the sliding angle of incidence, the light will propagate along the tubules under the conditions of the complete internal reflection. A thin section cut from the molar crown (the section perpendicular to the tooth axis), in its properties is similar to a fiber optic focusing cone made of glass fibers. A plate in the form of a thin section made from a bundle of dentinal tubules works a light concentrator. Such system has a small angular aperture, which transmits the test object image with different magnification/demagnification (Fig. 1). The test object is a plotting paper with the cell size of 1×1 mm.

The magnifying properties in different areas of the tooth thin section depend on the features of tubule inclination in the regional zones of the thin section [34,39–42]. When the thin section is illuminated with non-polarized light, the intensive background related to scattering is a noise for visual analysis of the local irregularities. To reduce the background when studying the optical properties of anisotropic disperse systems, such as tubules, it is useful to apply the polarized light. The interaction of the polarized light with the ordered structural zones of the thin section in the form of cone-shaped figures makes it possible to experimentally visualize the features of symmetry for regional zones of the thin section. This provides the additional information for analysis of the tooth crown shape formation and explains the ability of the tooth thin section to magnify/demagnify the elements of the optical image [36].

Samples preparation

The objects of the study were 36 intact teeth — the second and third molars of the upper and lower jaws extracted in patients for orthodontic reasons. The average size of the second molar crown is 9.4 ± 0.24 and 10.2 ± 0.24 mm for the upper and lower jaws, accordingly. The average size of the third molar crown is practically the same as the size of the second molar. The teeth samples had no caries or non-caries defects. The samples were made for two sections, when the plane of the thin section is perpendicular (horizontal section) and parallel (vertical section) of the tooth axis. After removal and fixation in 40% solution of formalin, thin sections were prepared for the samples in the form of parallel-plane plates with thickness of $\sim 1000\,\mu$m [35,36]. Some molar samples were prepared by orientation of the vertical section of the thin section passing through the tops of the tooth tubercles (mesio-occluso-distal). Thin sections of the teeth specified in the article were prepared by V.N. Grisimov, MD.

Justification of the polarization method

In case of ordered placement of anisotropic dentinal tubules around the center in the vicinity of the local regional areas such tooth sections will have the axial symmetry. The availability of the common center in the system of ordered waveguides, which is the bundle of dentinal tubules, may be visualized with the help of the optical method on the basis of crossed polarizers. When the linearly polarized light passes along the axis of the bundle, the phase shift, as the light passes through the bundle, will depend on the inclination of the tubules relative to the axis of the bundle. The light upon passage of the bundle with the axial symmetry of tubules location will be periodically put out by the polarizer (analyzer) and as a result, the system of concentric light and dark rings crossed with a dark cross may be visualized. The diameter of the rings depends on the thickness of the thin section and the structure of the tubules stacking in the bundle, which is reflected in the parts of symmetry-asymmetry of the visualized pictures.

The detailed description and the diagram of the experimental plant based on the crossed polarizers are specified in the paper [27]. Fine thin sections are used for the studies in a box with moist medium, which is placed between the polarizer and the analyzer. The optimal orientation of the thin section is specified visually so that the vector of polarization of the incident light matches the plane passing through the axis of the cone in the zone of the tubercle and the vertical line recovered in the center of the cone. The reproducibility of the visual observations of specific samples of the thin section is achieved by fixation of the thin section position relative to the vector **E** of the polarized light at the outlet of the polarizer. For this purpose, a mark is made on the thin section (for example, dents on the sample), the position of which may just be set within the limits of $\pm 2^\circ$.

The reproducibility of the photo for various samples of thin sections depends on individual features of tooth structure in a certain patient and is determined by the composition of the dentinal tubules microstructure in the regional sections of a specific tooth crown. When comparing the photos for 36 samples of molars, it was noted that for a small quantity of samples ($\sim 7\%$) in the corresponding regional zones, the size of which is $\sim 1-1.5$ mm, the specific typical patterns of images (crosses and concentric rings) were not observed practically. This indicates a spatially disordered and non-rectilinear (curvy) passage of dentinal tubules in these zones. For $\sim 75\%$ samples the regional local zones of thin sections demonstrated typical images: for the second molar 4(3) crosses and for the third molar 5(4) crosses. For individual samples the crosses were framed with concentric rings, the contrast of such pattern reduced further from the center of crossing. Since the nature of origin of the typical visualized image in the form of crosses depends on the microstructure of dentinal tubules, this gives reason to compare these structures when studying various teeth. For example, in the papers [27,36–38] the photos of local zones of molars and premolars are compared. To visualize the photo of the tooth crown thin section, it is necessary to make fine thin sections that restricts the area of using the optical polarization method. The specific nature of the method of crossed polarizers is related to the strong attenuation of light passing through the thin section, especially when the structure of the sample is homogeneous. In this case the contrast of the observed image significantly decreases. The restrictions are also imposed on the study of old teeth, because the thin sections of such teeth usually have low transparence and scatter light strongly, which decreases the contrast of the observed picture. At the same time the high sensitivity of this method, making it possible to study the features of the tooth growth on the basis of the image symmetry distortion analysis, makes the polarization method an important addition to other methods of study.

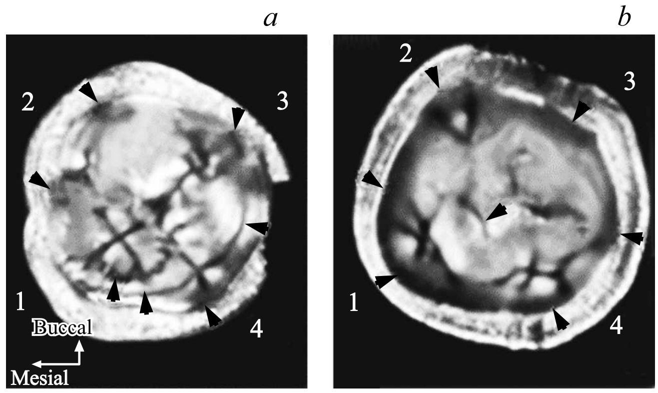

Figure 2. The photo (patterns) of the thin section produced for transmission in the crossed polarizers for two second molars (*a, b*), cut across the axis of the tooth (horizontal section). The scheme to make photos is shown in Fig. 3. Legend: mesial — mesial surface, buccal — vestibular (buccal) surface, 1–4 — areas of tubercles (growth zones of the molar). The arrows indicate „dark" lines and „darkened" areas in the vicinity of tubercles. „Dark" lines are specific for the pattern a, while „darkened" zones are more specific for the pattern *b*.

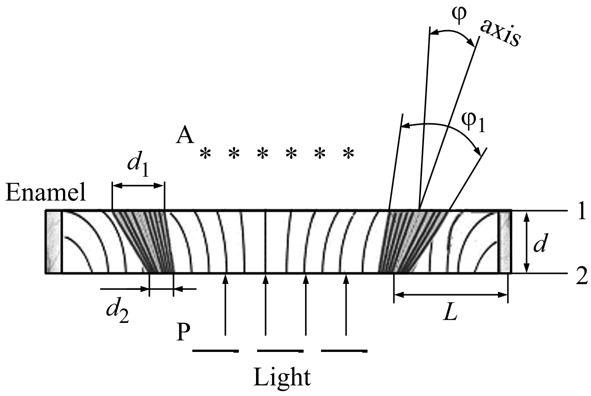

Figure 3. The scheme of the experiment and the structure of axisymmetric stacking of dentinal tubules for the horizontal section of the thin section in the area of the molar tooth crown tubercles: 1 and 2 — planes of the thin section (Fig. 1), d — thickness of the thin section, L — distance between the axis of the bundle of dentinal tubules and the outer border of the enamel, φ — angle of inclination of the axis of the bundle of tubules, ϖ_1 — angle of the bundle cross section, d_1 — diameter of the bundle in the area of the tubercle for plane 1, d_2 — diameter of the bundle in the area of the tubercle for plane 2. Arrows and stars indicate the positions of **E**-vectors of polarization when the thin section is illuminated. P — polarizer, A — analyzer. Direction of light incidence from the side of P, observation is performed from the side of A.

Diffusion method

To determine the permeability of thin sections, ions of dyes are used, which under external hydraulic pressure diffuse inside the dentinal tubules [32,46–48]. Permeability of tubules is the ability to transport pulpal fluid, which participates in the tooth biogenesis. Permeability of dentin first of all depends on the dentin thickness inside the thin section (i.e. length of tubules) and diameter of tubules. Since the tubules are shorter, more multiple and have larger diameter when closer to pulp, the depth dentin is a less efficient pulpal barrier compared to the surface dentin.

Regional differences in permeability of dentin are observed on the periphery with high values near the dentin-enamel layer (DEL) and low ones in the center of the crown thin section. Hydraulic conductivity of dentin in the radial direction from the center of the thin section reduces further from the pulp as the dentin thickness increases. Permeability of dentin may vary 3–10 times within several millimeters in different regional zones of the tooth [46–48].

Images of types of figure (patterns) under special staining of thin sections have been observed for quite a while, but no single classification of such zones has yet been proposed. As an example, one may note the general separation of such structures in the form of local textural and global features [46]. Therefore, for further study of regional (local) properties of teeth it is important to identify the features of the structural arrangement of tubules. Therefore, it is relevant to use various methods that would supplement each other informatively.

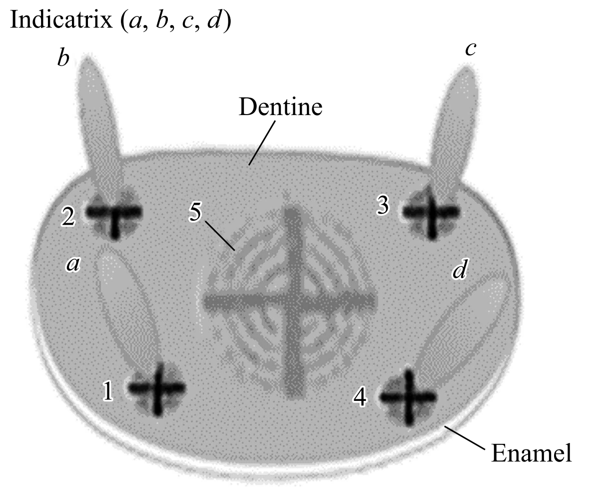

Figure 4. The model of the molar thin section with regular stacking of tubules relative to the axis of the bundle from the dentinal tubules in the zone of tubercles (1–4) and in the center of the thin section (5). Brightness indicatrices (*a, b, c, d*) are shown accordingly in every zone for the tubercles (1–4). The brightness indicatrix in the center is much weaker and is not presented. The scheme of the thin section illumination with the polarized light is shown in Fig. 3.

Results

Visual observations of the tooth crown thin section structure using crossed polarizers make it possible to obtain the additional information on the object properties. The features of the regional structure of the molar crown thin section are fully compliant with the properties of patterns, which makes it possible to compare it with the similar structures obtained by other methods. As an example, one may compare the patterns produced by two different methods: optical (in the crossed polarizers) (Fig. 2) and ion diffusion (Fig. 6) [16,32,46–48].

Types of figures produced by the visual optical polarization method demonstrate the elements of figure symmetry mostly in the form of deformed crosses. The shape of crosses displays the features of the structure of the regional zones of the thin section. The dimensions of crosses correlate with the height of the tooth tubercles. These data supplement well the patterns produced by the diffusion method and displaying the permeability of dentin tubules for the stained fluid in different zones of the thin section. In the schematic image of the dentinal tubules location it is common to represent the structural organization of tubules in the form of a combination of tubules with the symmetrical location relative to the center of the bundle. In the paper [49] it is shown that most tubules do not divert from the DEL at the at the right angle. The tubules always significantly change their orientation near the DEL both in the buccal and lingual sides of the premolars and molars. The tubules also tend to twist in this zone. At the same time the tubules for the lower jaw teeth are inclined normally in direction to the DEL with the average angle of 42°, while for the upper teeth the tubules show the inclination angle of 32° [49]. The properties of tubules and their size are related to the permeability for the fluid and sensitivity of dentin. The most structural differences are observed in the area of the molar crown, where dentin is more permeable than in the area of the tooth root. Therefore, for the visual comparison of the dentin properties obtained when using the polarization optical method and other methods, it makes dense to identify the orientation of the vector **E** of the light beam polarization.

The section of the thin section is perpendicular to the tooth axis (the vector E of the polarizer is parallel to the thin section plane)

When the molar thin section of the considered orientation is illuminated in the parallel light beam using crossed polarizers, sometimes one can see individual parts in the center of the thin section in the form of fragments of a large „Maltese cross“ [36]. However, the brighter and more specific figures of „Maltese cross“ type of smaller size may be observed much more often for the molar not in the center of the thin section, but at the border of DEL. Here the lighter zones are located, which contrast with the darker background for the center of the thin section [36–38].

Light zones On the photo of the tooth thin section (Fig. 2) one can see the light areas of growth in the area of the molar tooth tubercles that are often framed with comparatively narrow dark lines. The light zones are due to a combination of isolated dentin tubules organized in the

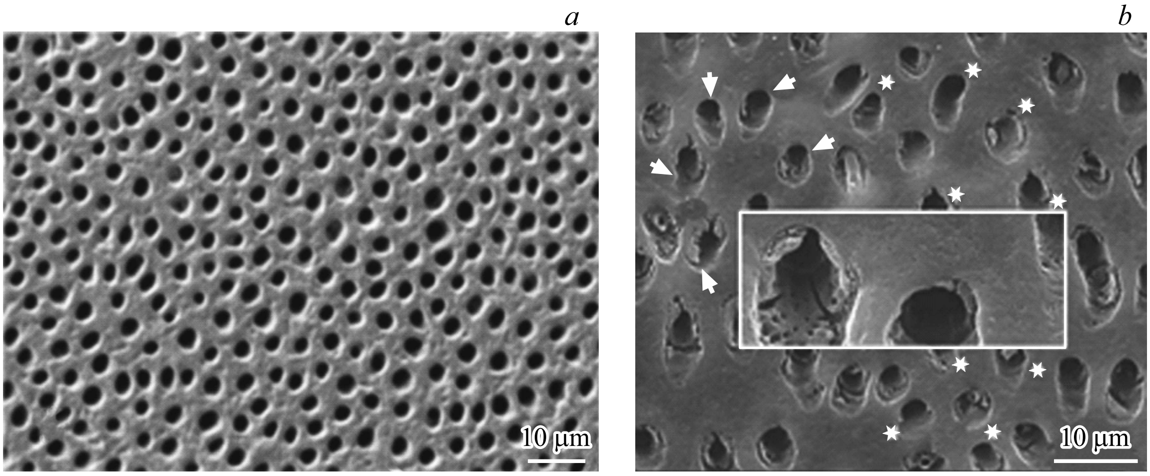

Figure 5. Images of dentin, the method of electronic scanning microscopy [25]. (*a*) The surface of the human toon dentin after grinding and mechanical polishing is additionally polished with the ion beam (FIB) [50]. (*b*) The polished surface of the bull incisor dentin in the vicinity of the growth zone. The arrows indicate the limited area that represents a local zone, around which an ordered structure is formed, which is symmetrical relative to the conventional center (local zone), formed by individual dentin tubules. The position of the center of the local zone having the shape of ellipse is determined by the point of crossing of its axes. The stars indicate the tubules that concentrate around the center of the local zone. The highlighted rectangular fragment with magnification of $3^{\times}$ compared to the main image shows the presence of fine openings inside PTD in contrast to ITD.

form of a cone with an axis directed from the tooth pulp and passing through the tooth tubercle top (Fig. 3).

Brightness of zones with figures in the form of crosses differs significantly from the overall yellow and green background and is usually more intense in the area of tubercles (Fig. 2,*a*). The light beams propagating along the tubules for these bright small zones have a small aperture, and the axes of these zones make the angle of $\sim 25^\circ$ normally to the surface of the thin section. The brightness of the separate zone increases greatly when observed along the bundle axis. For individual objects sometimes in the center of the thin section one may notice a figure with low contrast in the form of elements of a „Maltese cross" that is larger than the dimensions of the similar figures in the area of bright regional zones located in the area of molar tubercles (Fig. 4). The first visual observations in the crossed polarizers [36] made it possible to first observe the fragments of the low-contrast large ($\sim 4-6$ mm) „Maltese cross" in the center of the thin section inside quadrants 2, 3 with a series of concentric light and dark rings, which indicates uniaxial symmetry of tubules in the center of the molar thin section. Such samples with the fragments of the large „Maltese cross" in the center of the thin section sometimes occurred in our studies. Nevertheless, all these data indicate the availability of the elements of symmetry as the bundle of tubules forms in the center of the thin section.

The features of the observed figures (symmetry–asymmetry) for the tooth crown thin section are related to distortion of the system orientation of tubules in the regional growth zones that form a tooth tubercle. Some tubules have S-shaped configuration and show a curvature in different areas of the crown. The curvature of tubules in the area of the crown center (fissure) is generally shown in the form of stochastic curved branches spreading from the center. In contrast to such stacking, specific for the tooth center, in the area of molar tubercles the tubules are located systemically inside a relatively narrow conical bundle. Such stacking of tubules makes it possible to observe the figures with the elements of symmetry (Fig. 2). The diagram for the model of the tooth thin section (Fig. 4) shows that axes compliant with the bundles of tubules from different growth zones of the second molar meet in the area of the tooth pulp [27]. Different inclination of tubules in the central part of the tooth and in the vicinity of the growth zones of molar tubercles results in optical effects related to the deformation of figures in the form of crosses (Fig. 2). In particular, the differences of the regional (local) inclination of tubules relative to the plane of the thin section result in irregularity of the properties of test image elements magnification (Fig. 1) [32,37–40].

The areas of differences in the structural arrangement of tubules in separate parts of the tooth crown have been widely observed before with the help of the scanning electron microscopy [19,50–52]. In these papers the primary attention was paid to the numerical characteristics of dentin (diameter of tubules, density per unit of area, dependence of tubules on the distance to pulp etc.). At the same time, some papers [25,53] contain information that indicate the specific signs of the structural organization. For example, for the local area of the thin section of the tooth the arrows in Fig. 5,*b* show the relation of the tubules orientation with the elliptical cross section depending on their azimuth [25].

In Fig. 5, *b* one can see that around the conventional center ($\sim 15\,\mu$m), indicated with arrows, there are alternating concentric sequences of tubules with clear signs of organization [25].Such systemic nature of the tubule

Morphometric characteristics of the human molar crown thin section according to data of optical measurements, ion diffusion [32] and electrometric measurements [43–44]

Numbers tubercles	Area of growth zone/ cross section area of thin section, rel. unit.		Largest linear size of the main growth zone/section thin section, rel. unit			Diameter of inner of (open) ring/diameter of the main zone of growth rel. unit	Thickness inner (open) of ring, μm	Parameters of growth zones in the vicinity of tubercle		
	Study methods		Study methods			Diffusion of ions		Optical	Diffusion of ions	
	Optical	Diffusion of ions	Optical	Diffusion of ions	Electrometrical	Fine/ Large zone	Main growth zone	Number large zones	Number large and fine zone	Diameter fine zones μm
1	0.045	0.025−0.048	0.24	0.16	$\sim$0.18	$\geq$0.50/0.45	$\sim$80	1	1−$\geq$10	$\sim$350
2	0.026	0.016−0.025	0.16	0.14	$\sim$0.16	$\geq$0.50/0.45	$\sim$41	1	1−$\geq$10	$\sim$350
3	0.020	0.015−0.020	0.14	0.10	$\sim$0.14	$\geq$0.50/0.40	$\sim$20	2	3−$\sim$11	$\sim$200
4	0.010	0.010−0.018	0.10	0.08	$\sim$0.14	$\geq$0.50/0.41	$\leq$15	2	1−$\sim$12	$\sim$200
5	–	$\leq$ 0.01	–	$\leq$ 0.1	–	$\geq$0.50/0.45	$\leq$8	–	0−$\sim$4	$\sim$100

Note. As an example, the table for two individual samples includes approximate parameters calculated on the basis of optical and electrometric measurements [43–44] for the second molar and diffusion of ions (for the third molar) [32]. For the method of ion diffusion, the parameters of 3−5 zones of the molar formed near individual smaller zones were estimated by averaging the dimensions of these small zones. The estimate of the area of growth zones (rel. unit) for the ion diffusion method is given for two limit cases: the least area corresponds to the area of the main zone — local zone with maximum blackening (Fig. 6), and the largest area corresponds to the area including the local round areas of small size that are adjacent to the maximum blackening area.

structure indicates the presence of the center inside such composition.

„Dark“ lines and „darkened“ zones For the process of tooth formation, the so called „dark“ lines and „darkened“ zones matter, which are specified by the arrows on the patterns (Fig. 2). The dark lines and the darkened zones are mostly grouped between the DEL and the main growth zone in the center of the tubercle. The lengthy dark lines are formed by closely located bundles of small diameter ($\sim 100-350\,\mu$m), which consist of the dentinal tubules with the angle of cone $\varphi_1 \leq 1^\circ$ (table). These narrow bundles are formed by a system of practically parallel anisotropic tubules, with the beam S_o put out by the analyzer when passing through them. If the image dimensions are small, the figure in the form of a „Maltese cross“ together with the system of rings may not be formed due to limited dimensions of the optical beam. One may compare the obtained data with the hydrostatic method of dye ion diffusion in the patterns (Fig. 6) [16,32]. From this comparison it follows that the narrow bundles of tubules closed between each other that are observed in the form of dark lines and darkened areas, represent small local growth zones located around the main growth zones that form the tooth tubercles.

The functional purpose of the small regional growth zones consists in the correction of relief when an individual tooth tubercle is being formed. Therefore, the patterns of the tooth crown produced by the optical method (Fig. 2) may quite be compared to the patterns (Fig. 6) produced by the hydrostatic method of diffusion, where the regional main and local growth zones are well seen. The analysis of the dark lines obtained on the basis of the optical measurements of different thickness in the vicinity of tubercles makes it

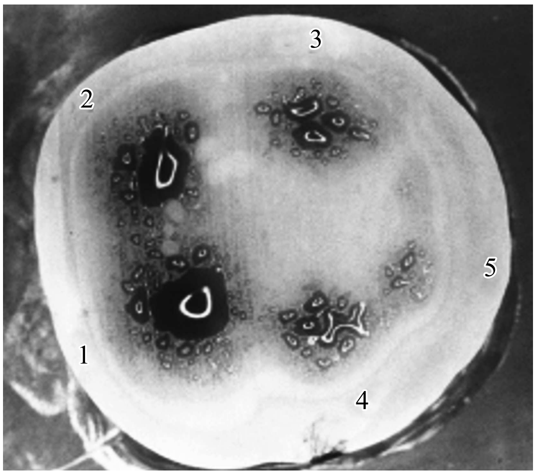

Figure 6. The horizontal slice of the molar crown (patterns) [32]. The fluid with dark blue dye was injected into the pulp chamber under pressure. Dark areas of dentin in the area of tubercles (above the pulp horns) are much more permeable than the surface (unstained) dentin in the center of the thin section. White unstained (open) rings are located in the center of tubercles (dark area), where dentin is not permeable for the dye. Legend: (1−5) — areas of tubercles (growth zones) of the third molar thin section.

possible to independently assess the averaged size of the fine growth zones, which is $\geq 100\,\mu$m, being compliant with the data for the diffusion method (table). Therefore, both methods supplement each other and help to study the formation of the mammal teeth [7], thus demonstrating the potential of these methods to study the shape and lateral growth of the tooth tubercles.

Zones with „Maltese cross“ Regional areas of the thin section, where the figures of „Maltese cross“ type are observed, are formed by zones in the form of a cone with regular stacking of anisotropic tubules. If such anisotropic tubules are linear and form a bundle in the form of a cone, the light passing through such cone via crossed polarizers will have the axial symmetry [36–38]. With account of the anisotropy the bundle of tubules will be characterized at the exit with the phase difference δ for two noninteracting beams: ordinary S_o and non-ordinary S_e. The phase difference δ for these beams stacked in the form of a cone will follow the rule of radial symmetry. It is material that the beams exiting certain linear tubules at a difference distance from axis ρ and azimuth $\alpha = 0{-}360°$, will be elliptically polarized. Phase shift δ for linear tubules will depend on the parameters d, φ, ρ, n, Δn and α [27,38]. The number of reflections N of the beam inside the tubules will depend on these parameters. If the tubules have non-linear shape, for example, S-shaped appearance, and may twist along the axis, the value of phase δ will additionally depend on the following parameters: β — parameter of tubule twisting and ρ — cone radius ($\rho_{\max} = d_1/2$).

The following parameters were used for the bundle of linear tubules when calculating the phase δ: d — thickness of the thin section, d_{ext} — external diameter of tubles, $n = n_{\text{ITD}}/n_{\text{PTD}}$ — relative refraction index of dentin, $\varphi(\rho)$ — angle of inclination of the tubules in the bundle, φ_1 — angle of cone cross section and parameter $\beta = 0$

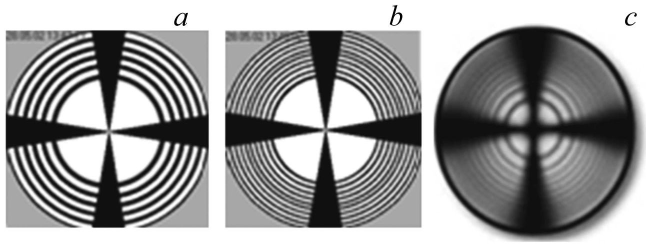

Figure 7. Calculation (a, b): the appearance of the interference picture (patterns) for simulation of the molar thin section cut across the axis of the tooth (horizontal section, Fig. 3), monochromatic light, $\lambda = 0.55\,\mu$m, polarizers P and A are crossed [38], internal diameter of tubules $d_{\text{int}} = 0.1$ (a), $1.5\,\mu$m (b), external diameter of tubules $d_{\text{ext}} = 3.5\,\mu$m, thickness of thin section $d = 1000\,\mu$m, $\varphi = 10°$, $\varphi_1 = 10°$; dark rings demonstrate the areas of the phase difference $\delta = 2\pi + \pi$ etc. at the exit of the tubules; figures in the form of crosses and dark zones — areas of beam suppression. (c) Experiment: classic conoscopic picture in the converging light beam for the uniaxial crystal (thickness 1 cm), cut perpendicularly to the optical axis.

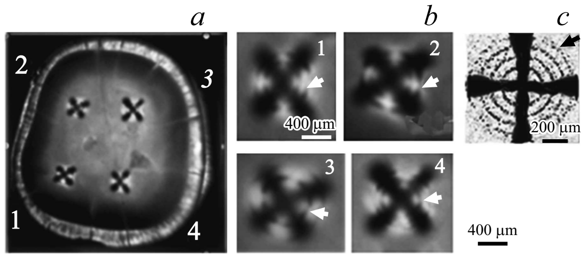

Figure 8. Experiment: (a) photo (patterns) of the second molar thin section cut across the tooth axis, obtained for transmission in the crossed polarizers (growth zones 1–4); (b) magnified images of the growth zones 1–4, the arrows indicate the interference rings framing the central part of the crosses; there are breaks in the rings illumination seen in the central part of quadrants. The absence of the rings further from the center specifies that the regular axisymmetric stacking of tubules for the growth zones 1–4 is maintained mostly closer to the crossing (axis of the bundle of tubules) and varies for different areas. (c) For comparison, the local zone of the premolar tooth is provided [38] with the regular axisymmetric stacking of linear tubules.

(Fig. 7). Software was developed for the corresponding calculations, which makes it possible to demonstrate the influence of certain parameters of dentin organized in the form of an axisymmetric cone from tubules, the axis of which is arranged at a small angle to the incident beam of light [38]. For the typical conditions: $\mathbf{E}$ — vector of the polarizer is perpendicular to the axis of the cone, then at the entrance to the tubules only one beam S_o will propagate, which will be put out by the analyzer at the exit from the cone. In the center of the calculated pattern, a cross will be observed, the crossing of which matches the axis of the cone.

The shape of the cross somewhat differs from the typical „Maltese cross“, which is observed in the experiment when studying the thick uniaxial monocrystals. It is related to the fact that the conditions for putting out the light for the system of tubules parameters adopted for the calculation are met only in direct vicinity for the main directions of $\mathbf{E}$-vectors of the polarizer and the analyzer. Another specific feature of the estimated pattern — appearance of dark and light rings crossed by the cross in the center. Appearance of the ring is due to the fact that the phase difference δ depends on the ratio ρ and azimuth $\alpha = 0{-}360°$. The first dark ring from the center corresponds to the phase difference $\delta = 2\pi + \pi$ etc. It should be noted that all observed parts of the described pattern are specific for the axisymmetric stacking of the bundle of linear tubules in the form of a cone. If the angles of inclination of the tubules in the bundle are of stochastic nature, one can only see the image of the cross in the center of the bundle, and the rings will not be observed.

The presence of the rings in the experiment indicates the ordered arrangement of the tubules around the axis of the cone (Fig. 8). Most contrast figures in the form of crosses are observed for the two positions of the crossed polarizers, while the direction of the **E**-vector of the incident light forms a perpendicular line with the direction of the axis of the truncated cone.

Appearances of the patterns' rings near the center of the cross figure and their absence further from the center is related to the distortion in the axisymmetric stacking of anisotropic tubules (Fig. 8, *a, b*). Irregular stacking of tubules changes the systemic nature of the function $\delta(\rho, \alpha)$, at the same time one may note that the value of distortions depends on the distance ρ further from the cone axis. From patterns (Fig. 8, *a, b*) one can see that the deviation of the phase δ at all values ρ is highest for azimuths α, corresponding to the position of diagonals inside each of four quadrants. This indicates that the primary bundle from the polarizer P after passage inside the tubules is depolarized the most for this direction α. In other words, two beams S_o and S_e will propagate in this direction. This feature of irregular distribution of illumination inside the contour of the rings demonstrates the difference between the calculation of Fig. 7 with account of the performed approximations and patterns of Fig. 8. One may note that the frequency of patterns' rings may not be maintained for the adjacent quadrants (Fig. 8, *b*). This is due to the fluctuations of the phase $\delta(\rho, \alpha)$ as a result of irregular linear dimensions of the tubules in the vicinity of a certain quadrant or local rotation of S-tubules in this quadrant.

Concentric rings for the studied batch of thin sections were observed only in one or two-three quadrants in individual samples of thin sections, which indicates systemic stacking of dentinal tubules in the vicinity of these quadrants. It should also be noted that there were samples, for which the figures were observed in the area of regional zones, which were different from the crosses (remind of the letters V or X, open eights, three-pointed stars) [36–38].

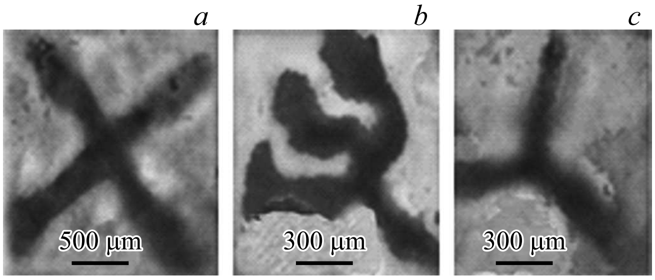

Figure 9. Experiment. Typical patterns of the thin section fragments from different samples of the second molar produced in crossed polarizers. Fragments characterize the regional growth zones differing by the stacking of tubules: (a) cross, the tubules have linear shape, the stacking of tubules relative to the center is ordered; (b) trident, some tubules (see the upper left corner) on the periphery of the growth zone are twisted along the axis of the bundle, these are tubules twisted or bent in the form of letter S; (c) three-pointed star, the growth zone is formed from three independent local sections of growth (for example, the growth zone 3 in Fig. 6 and 10, *b*), in this case the bundles of tubules of each of three sections have their differing polarization parameters, mutually aligned at angle 120°.

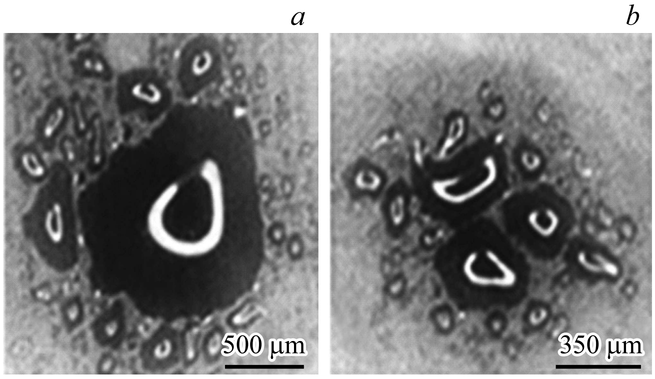

Figure 10. Growth zones 1 (*a*) and 3 (*b*) of the horizontal thin section of the third molar (patterns) (Fig. 6). It is interesting to note that the break areas for the unstained open rings are oriented in the same direction and correspond to the growth zones of dentin that are impermeable for the dye.

Figures in the form of the letter V (for example, the growth zone of patterns 3, Fig. 2, *b*), the letter X (for example, in the patterns the growth zone 4, Fig. 2, *a*) and the open eight are formed in case of strong asymmetry in the image of the „Maltese cross" for the two adjacent quadrants (V) or two opposite quadrants (X, open eight).

The availability of the adjacent local grown zones having one symmetry may result in merger of these zones manifesting itself in the form of joined centers forming crosses (patterns, Fig. 2). For example, in the periphery in the area of growth zones 1 and 2 (Fig. 2, *b*) one may often note a combination of two figures reminding of the deformed crosses. Deformation of the cross form may be caused by the features of twisted tubules stacking. For example, the observed pattern may be related to appearance of S-tubules twisted along the axis, besides, according to the patterns in Fig. 2, *a* and 9, *b*, the twisting angle increases gradually further from the center of the figure. Twisting of S-tubules results in weaker contrast of the rings inside every of four quadrants.

Figures that are more complicated for interpretation (Fig. 9) are formed, for example, when the figure is observed in the form of a three-pointed star.

This case may be explained based on the assumption that there are three interdependent local growth zones nearby (for example, growth zone 3 in Fig. 6), which have a common regulating center located in the vicinity of the tooth tubercle. To form a three-pointed star, it should be taken into account that the tubules are S-shaped and may twist [13–15]. In each of the individual three growth zones a bundle of tubules of homogeneous structure is formed, which is turned towards the adjacent zone by 120°. At the same time for each of the bundles at the exit we only have beams S_o, which are put out by the analyzer. Such synchronous connection of the local growth zones in the vicinity of one tubercle is compliant with the hypothesis about the induction interaction of the growth dentin cells —

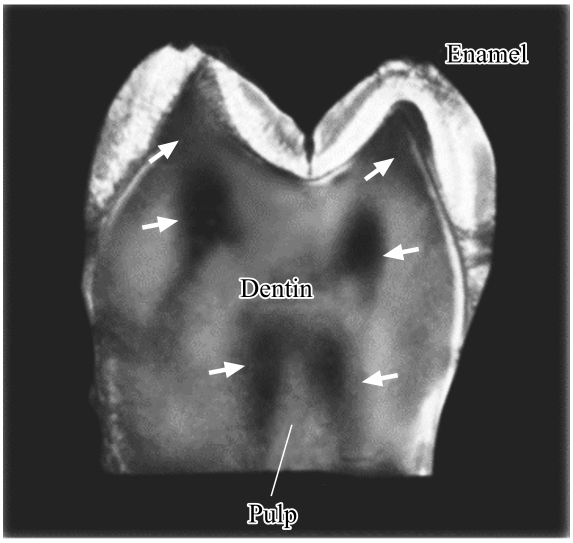

Figure 11. The photo (patterns) of the second molar thin section cut along the tooth axis (vertical section), produced in the crossed polarizers. The arrows indicate the dark fragments of the elongated shape stretching from the pulp in direction of the central part of the tooth tubercles.

odontoblasts, which may be controlled via specialized nerve cells located inside tubules [20–24].

Different dimensions of the local growth zones in the vicinity of the molar tubercles produced with the help of the ion diffusion method are shown in Fig.10 [16,32].

Morphometric estimate of these growth zones characteristics are generalized for three independent methods of measurements (table). From the table analysis one may see a good correlation between the estimates of the growth zones dimensions produced by the comparable methods. At the same time for the diffusion method there are additional substantial parts in the form of open light rings, which are absent in the patterns for the optical method (compare Fig. 2 and 6, 10). The presence of the open light rings in the center of the growth zones (Fig. 6) indicates the absence of the pulpal fluid flow inside this zone. Dimensions of the diameters of small growth zones are in the interval of $\sim 100-350\,\mu$m (table), which, according to the estimate, corresponds to 400-5000 tubules inside small growth zones. Breaks of the transparent rings have the same orientation. The position of the zone center produced from the data of the hydrostatic method of dye ion diffusion (Fig. 10) matches the center of the growth zone for the patterns produced by the optical method and complies with the position of the crossing in a specific zone (Fig. 2). The area of the transparent rings in the center of the growth zone may not be observed visually, since the optical properties of the tubules in this area practically do not differ from the peripheral tubules of the growth zone. The coincidence of the listed properties in the compared zones (Fig. 2 and 10) makes it possible to assume that inside a site limited with an open transparent ring there are dentin tubules plugged with the nerve endings. The relative averaged diameter of the open ring for all growth zones remains constant (table). The thickness of the open transparent rings varies slightly for all tubercles and makes $\approx 10-80\,\mu$m, which makes it possible to estimate the number of tubules, which may be located inside a transparent ring within $\approx 2-16$. The presence of such specialized tubules in the center of the growth zone is to be logically connected to the nerve cells, which manage the growth of dentin tubules and form the shape of the crown relief in the vicinity of the local tubercle.

The section of the thin section is parallel to the tooth axis (the vector E of the polarizer is perpendicular to the plane of the thin section and tooth axis) The optical scheme of the molar thin section observation for the selected orientation in the transmission mode may be compared to the method of fiber study in the immersion medium. Anisotropic fibers (dentine tubules PTD) are in the immersion medium, which is the intertubular dentin (ITD), filling the gaps between the tubules. Since the refraction indices of PTD and ITD are close to each other, such composite system transmits the light beam well and relatively weakly scatters light.

Therefore, the use of crossed polarizers in the study of a thin section cut in parallel to the axis of the tooth makes it possible to observe a contrast picture in the form of light and dark fragments (Fig. 11). Comparison of these data

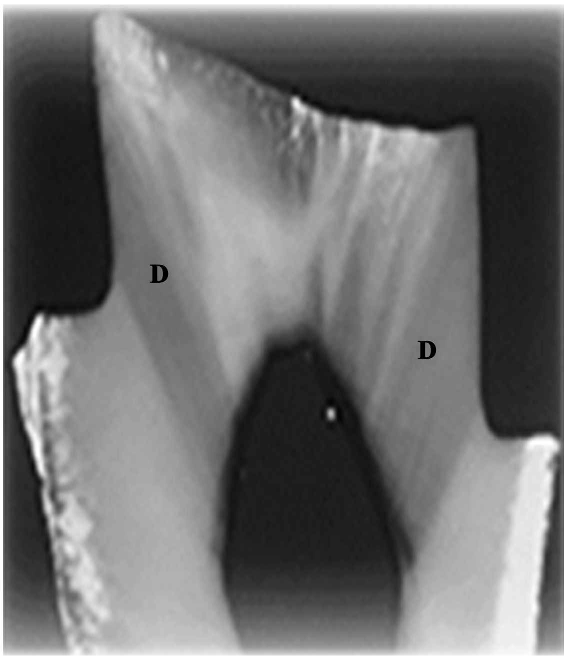

Figure 12. Polished slice of the modal (mesio-occluso-distal) tooth on the third molar. The dark blue dye was placed in a pulp chamber under pressure after the tooth preparation [13]. Dark areas of dye (D) penetration indicate that dentinal tubules of axial walls are much more permeable than those bordering the pulp. The dark oval area in the lower part of the figure is the area of the pulp which is well permeable for the dye.

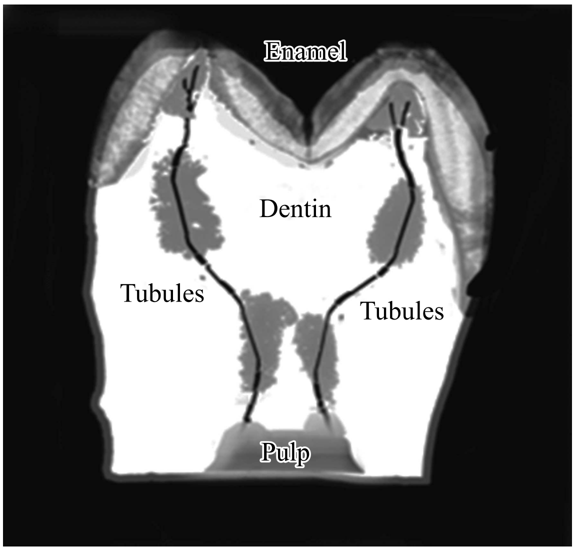

Figure 13. The scheme of the molar thin section simulating the patterns in Fig. 11. On the left and on the right from the central axis of the tooth there are solid dark curves (tubules), which illustrate the curved S-bundles made of anisotropic tubules. The scheme highlights the non-transparent areas of the thin section that correlate with the areas of curvature of S-bundles of anisotropic tubules (Fig. 11). Dark areas in the vicinity of S-bundles comply with the directions perpendicular to the vector **E**.

with the experiment for the ion diffusion of ions (Fig. 12) shows the differences in the contrast of the observed areas of dentine and specific nature of arrangement of the tubule bundles. The discontinuity of dark fragments (Fig. 11) is related to the configuration of the S-bundles made of a combination of anisotropic tubules that bend in direction away from the pulp to the top of certain tubercles of the tooth. Comparison of the direction of such S-bundles, which have a dark tone due to staining in the process of dye (**D**, Fig. 12) diffusion, shows that the bundle in the left part of the crown is homogeneous along the axis, while the wide bundle (**D**) on the right is supplemented with a series of narrow curved (S-bundles) separated with light gaps.

When the direction of the vector **E** is perpendicular to the identified area of the S-bundle, when the polarized light passes through this area of the bundle, the direction of vector **E** will not change. Therefore, the beam S_o that passed through the thickness of the S-bundle will be put out by the analyzer, which results in formation of dark areas on the photo.

In contrast to the considered example, for the area of bend of the S-bundle the vector **E** of incident light will make a sharp angle with the axis of the bundle. As a result, two beams S_o and S_e will appear, the light that passed through this area will have the phase difference δ and will be elliptically polarized. As a result, such light that passed through the thickness of the tubule will be only partially put out by the analyzer. The model scheme (Fig. 13) shows the features of bend of two S-bundles made of anisotropic tubules.

Fig. 13 shows the areas of bend of two S-bundles. Dark areas and areas with the light background are formed along these bundles, with the background being of the same intensity as the background from dentin scattering. Both S-bundles maintain the bend pitch in direction from the pulp to the area of the tooth tubercles. The symmetry of arrangement of S-bundles along their axis matches well the patterns in Fig. 2 for the horizontal section of the tooth. The light areas in the other zones of the tooth are due to the combination of S-bundles with disordered structure.

Alternation of light and dark fragments located along the axis of the S-bundles makes it possible for the thin section cut perpendicularly to the axis of the tooth to independently assess the linear dimensions of the dark areas that form the growth zones of the tooth tubercles. The dimensions of these areas correlate with the main values of the bend bundles pitch and their diameters, which may be assessed for another thin section cut perpendicularly to the axis of the tooth. Besides, one may study the pitch of twisting of S-tubules at a different distance from the tooth pulp and compare the parameters of the adjacent bundles. For different teeth, based on the features of symmetry of the growth zones in the area of tubercles, the patterns in Fig. 2, *a*, *b* may be compared to Fig. 11. From this comparison it follows that for different horizontal sections in the area of the tooth crown the patterns may differ in details.

The completed studies show that the dentin structure in the vicinity of certain tubercles of the human molar teeth is formed by a combination of dentinal tubules, the so called growth zones, which are characterized by the axisymmetric stacking of tubules. The signs of asymmetry in the stacking of tubules specify the changes in the form of a certain tubercle, which makes it possible to study the patterns in formation of multi-root human teeth. The presence of dentin structure symmetry in the area of certain molar tubercles gives reason for the assumption on the connection of such structure with the structure of the primary conical teeth. Based on this fact, one should assume that the presence of asymmetry in the zone of certain molar tubercles is related to the processes of phylo- and ontogenesis, as a result of which the interdependence appears in the structure of the crown shape and the tooth crown being universal for all teeth of the class Mammalia [6,11]. Therefore, use of the crossed polarizers reduces scattering from the irregular dentin structures and makes it possible to study the ordered structures that demonstrate the features of symmetry provided for by the type of tubule stacking. The proposed approach to the study of the dentin structure may help to obtain the additional information related to the genesis of the formation of regional zones in the tooth crown.

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