From: The Department of Oral Histopathology, School of Dentistry, University of Lund, Malmö, Sweden.

A NEW METHOD OF MICROPHOTOGRAPHY OF DENTAL ENAMEL IN POLARISED LIGHT

by
ANNA-GRETA GUSTAFSON

INTRODUCTION

The double-refracting effect of crystals and crystalline substances is due to the internal pattern of the structures. The effect is visible in the polarising microscope, but as the extinction is dependent on the direction of the double-refractive substances and their relation to the vibration planes of the microscope, the full effect can only be seen if the microscope stage is rotated enabling the different parts of a structure to have their double-refraction evaluated. While photographs are being taken it is not possible to rotate the specimen stage at the same time, thus photographs taken in polarised light contain dark areas stemming from two different origins, namely: the areas without double-refractive substances and areas in which the vibration planes of the double-refractive substances are parallel to the vibration planes of the polarising filters or perpendicular to the specimen plane.

In the dental enamel the crystallites are strongly negatively birefringent and the effect should therefore be easily recognized in the microscope. However, since they follow practically the

same course as the enamel prisms and as these lead in different directions, some of both the crystallites and of the prisms, or part of them, are extinct because they lie parallel with the vibration planes of the microscope. Thus on a microphotograph some areas will appear dark although they contain a great number of crystallites parallel with the plane of the specimen. It is then difficult or practically impossible to demonstrate on a microphotograph the areas of hyper- or hypomineralisation, as they are partly hidden by the effect caused by bending.

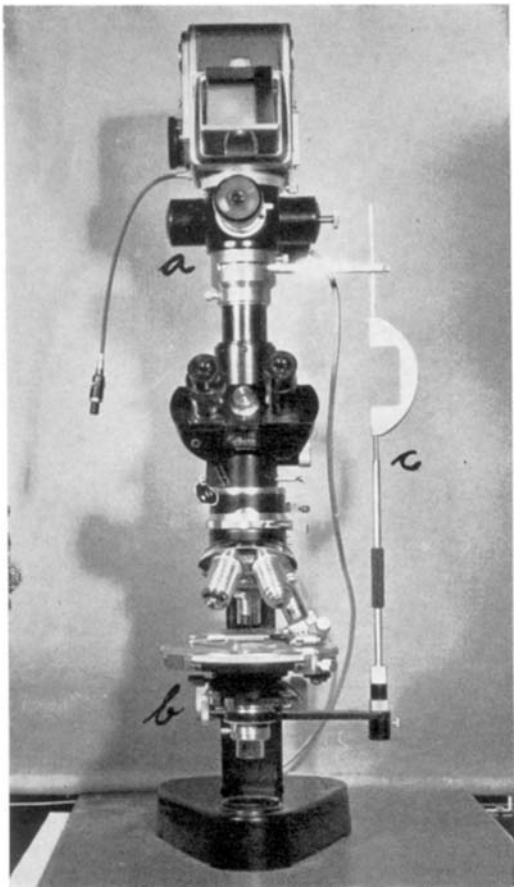

Fig. 1. A polarizing microscope where the analyser (a) is placed on top, instead of below the ocular, and combined with the polariser with the aid of a bar (c).

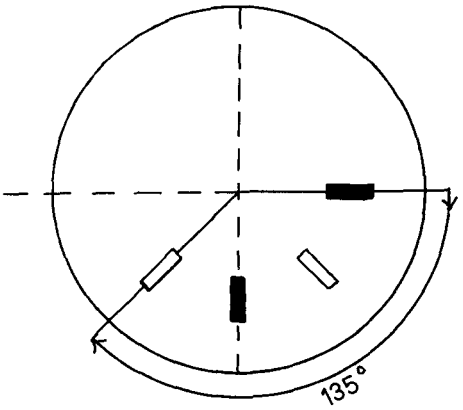

Fig. 2. When the combined filters are rotated from 0 to 135 degrees during exposure time, the birefringent particles are twice in extinction or masking position and twice in diagonal position.

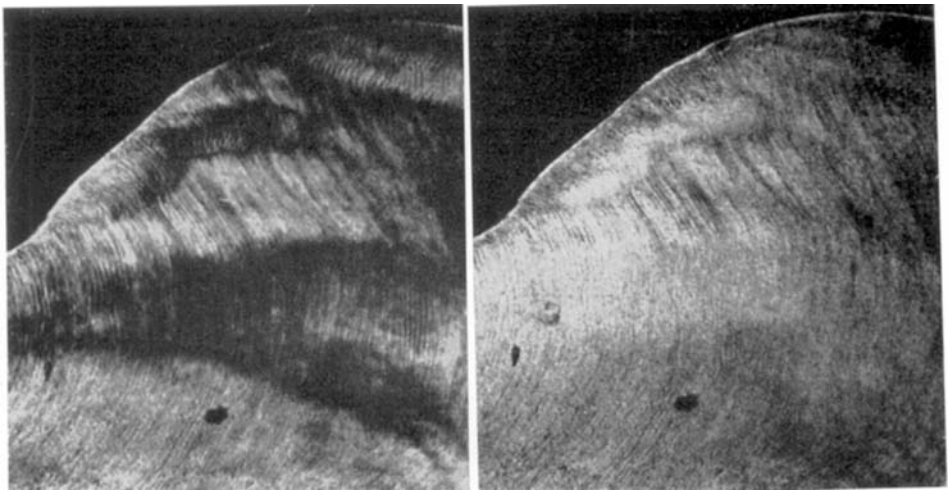

Fig. 3. a. The cusp of a molar photographed with filters; b. the same area with rotating filters during exposure showing prisms with equal degree of birefringence.

METHOD

In order to overcome this technical difficulty, the following device was constructed and attached to the polarising microscope to allow rotation of the polarising filters during the exposure.

The analyser, usually sited below the ocular, was placed on top of it and below the attached camera (Fig. 1). The analyser

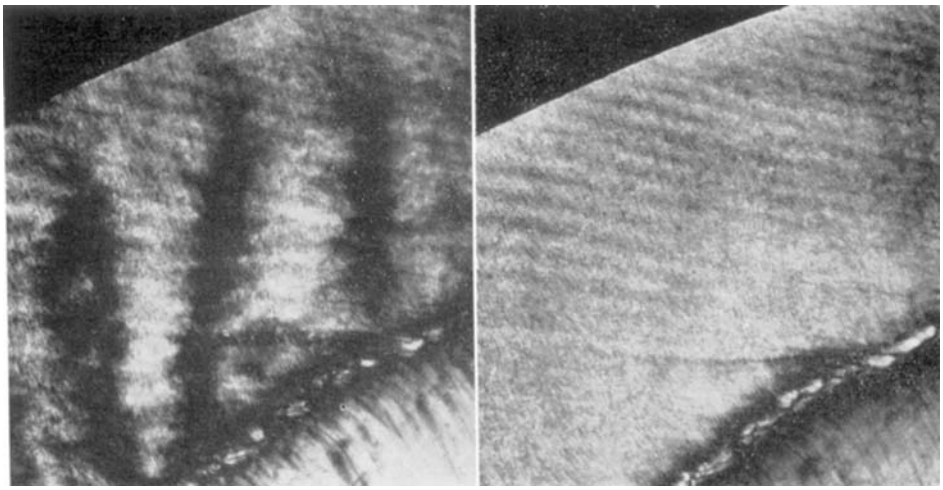

Fig. 4. On the microphotograph (a) taken with fixed filters the Retzius-lines are obscured by bendings of the prisms, on b, however, taken with rotaring filters, this phenomenon is eliminated and only real changes in structure are visible.

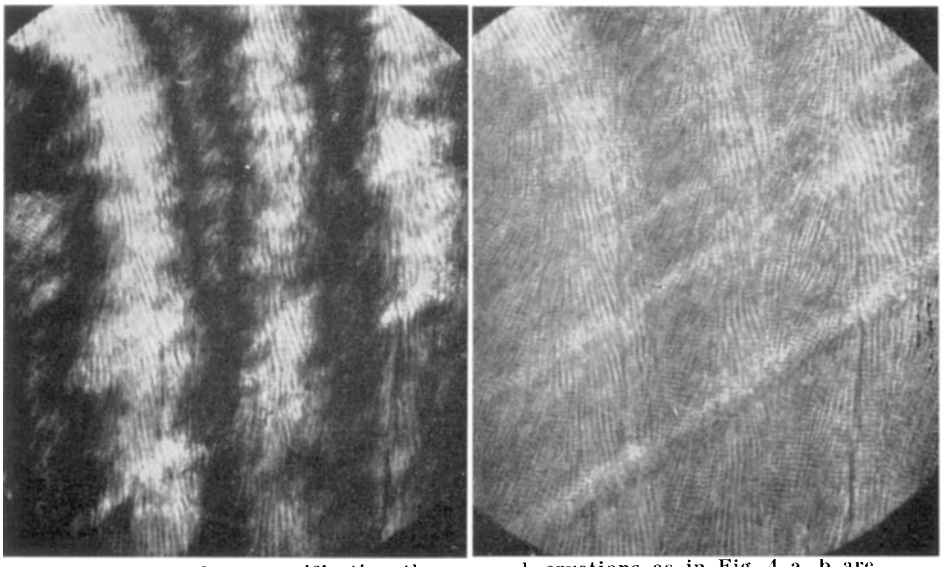

Fig. 5. In higher magnification the same observations as in Fig. 4 a, b are still more obvious. In Fig. 5 a (fixed filters) the difference in birefringence is evident whereas in Fig. 5 b, with rotating filters, a clear view of the Retzius-lines and the cross-striation comes into sight, as the effect from the bendings of the prisms is eliminated.

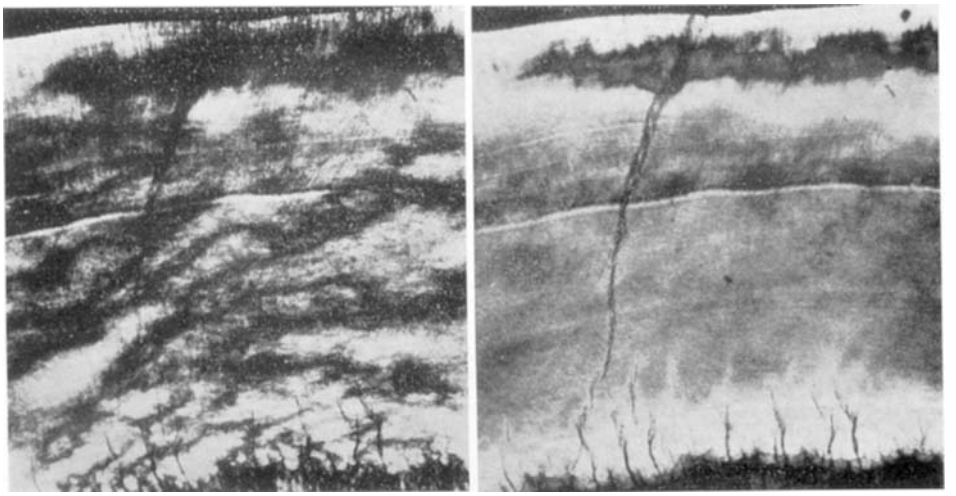

Fig. 6. From an ordinary microphotograph in polarised light as seen in Fig. 6 a, no clear information about the structure can be obtained; in Fig 6 b, however, with rotating filters a more adequate image is available. The Retzius-lines are clearly seen and the area with low mineralisation beneath the enamel surface is here strictly delineated.

(a) and the polariser (b) were combined with each other with the aid of a bar (c) so that they could be moved simultaneously with the polarising planes remaining crossed.

A slow film was used and the microphotographs taken while the combined filters were rotated from a zero position to 135 degrees from it. With the slow film and adequate lighting it was possible to expose for 3 to 10 seconds. Hence the areas containing birefringent particles in the plane of the specimen were twice in the extinction, or in the masking position, and twice in diagonal position giving maximal effect, or partially between these two extreme positions (Fig. 2). In the prototype the filters were moved manually but a motor control will in the future be attached resulting in the light being automatically switched on as the rotation starts.

FINDINGS

The areas covered by the following microphotographs represent, firstly (a) those taken with the polarising filters crossed but without movement during the exposure and secondly (b) with the filters rotated simultaneously 135° during exposure.

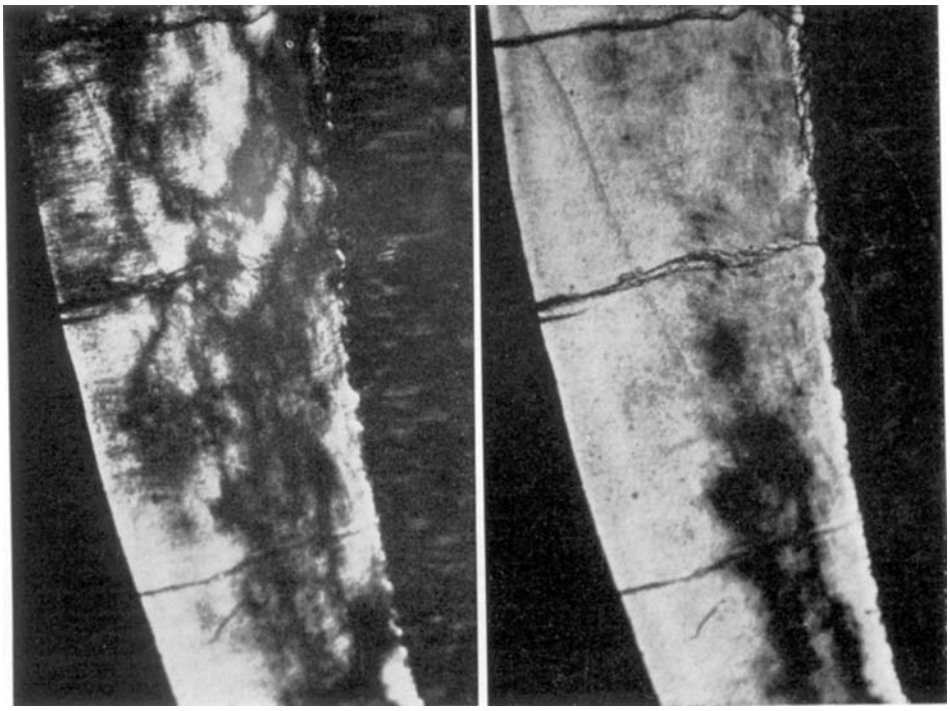

Fig. 7. A microphotograph from the neck of a tooth shown in Fig. 7 a appears rather confusing whereas in Fig. 7 b the same area taken with rotating filters presents more and better details. The Retzius-line, practically invisible in Fig. 7 a is here easy to discover.

In Figure 3 a the cusp of a premolar is photographed and as the filters remain fixed, all those areas, where the prisms and also the double-refracting crystallites are more or less parallel to the polarising planes, remain dark. It is thus impossible to see if certain parts contain prisms with low degrees of birefringence. In Figure 3 b the same area is photographed with rotating filters during exposure, and it appears possible that most of the area contains prisms of an equal degree of birefringence, but with changing directions.

Retzius lines, when present on the side of the teeth, are often obscured by bendings of the prisms so that in microphotographs with fixed filters (Fig. 4 a) the variations depending on bending are dominant, whereas when the filters are rotated (Fig. 4 b) the variations due to real changes in structure are clearly visible.

This point is made more obvious with higher magnification (5 a and 5 b) where in the first type of photograph (Fig. 5 a) the difference in birefringence is exaggerated. In the photograph taken with rotating filters more details are visible (Fig. 5 b), giving a clear view of the Retzius-lines and also of the cross-striations of the prisms.

When there is great variation in structure (and in birefringence), its nature and composition are not truthfully revealed by the ordinary microphotograph in polarised light (Fig. 6 a). This, however, is no longer a problem when filter rotation is used (Fig. 6 b). The Retzius-lines in the middle of the enamel are clearly seen and the area beneath the enamel surface is strictly delineated and since it can be shown, in higher magnification, that the prism here are practically parallel with the specimen plane, there must be an area with low negative birefringence and thus low mineralisation.

Identical findings can be demonstrated in Figures 7 a and 7 b where a hypomineralised area near the neck of the tooth is seen in Figure 7 b, but barely visible in Figure 7 a. Another example is the prominent Retzius-line in Figure 7 b which is practically invisible in Figure 7 a.

DISCUSSION

Naturally, those prisms which are perpendicular to the specimen plane do not change their apperance since they appear dark in all positions. But as can be seen from Figs. 3—7, it is of great advantage to obtain the effect of the birefringent particles originally parallel to the plane. This is particularly evident in those places where the prisms are parallel to the specimen plane.

Microphotographs of bone in polarised light are also more clear when taken with rotating polarising filters although in bone the structural pattern is very complicated and more difficult to interpret than in the enamel. The rotating-filter approach furthers the accuracy of structural assessment.

It is not possible to use the polarising microscope for this method with the analyser below the ocular since resulting photographs will be blurred due to a displacement of the image on the photographic film. This in its turn is directly related to the fact that the analyser is not accurately centred and the light is dis-

placed as it passes the planoparallel filter tilting a little against the direct path of the light. The resulting displacement is magnified by the ocular and the photographs become blurred and distorted.

SUMMARY

With the ordinary polarising microscope it is not possible to take microphotographs showing birefringent particles if they lie parallel to the vibration planes of the polarisers. Thus it is also not possible to evaluate the amount or the character of birefringent substance from such microphotographs. This paper describes a device which when attached to the microscope, allows a simultaneous rotation in 135° of the crossed polar during the exposure. With this it is possible to show all birefringent substances lying in the plane of the preparation.

RÉSUMÉ

UNE NOUVELLE MÉTHODE POUR LA MICROPHOTOGRAPHIE DE L'ÉMAIL DENTAIRE EN LUMIÈRE POLARISÉE.

L'effet de double réfringence est visible au microscope polarisant; mais l'extinction dépend de la direction des substance biréfringentes et de leur relations avec les plans de vibration du microscope; de ce fait l'effet total ne peut être observé que si la platine du microscope tourne de telle sorte que l'on puisse évaluer la double réfringence de toutes les portions de la structure. Quand on prend des photographies, on ne peut pas faire tourner la platine et ainsi les photographies prises en lumiére polarisée comportent des surfaces sombres, auxquelles on reconnaït deux origines: ce sont d'une part les surfaces ne contenant pas de substances biréfringentes, et d'autre part, des zones dans lesquelles les plans de vibration des substances biréfringentes sont parallèles aux plans de vibration des filtres polarisant ou perpendiculaires au plan de l'échantillon qu'on examine.

Pour améliorer la technique nous avons fixé sur le microscope polarisant une système qui permet la rotation des filtres polarisants pendant l'exposition, que peut durer 3 à 10 secondes. L'effet est demontré d'une part avec des filtres croisés mais immobilisés (a), et d'autre part au cours de la rotation simultanée de 135° pendant l'exposition (b).

ZUSAMMENFASSUNG
EINE NEUE METHODE FÜR MIKROPHOTOGRAPHIE DES ZAHNSCHMELZES IM POLARISIERTEN LICHT

Die Wirkung der Doppelbrechung kann im Polarisationsmikroskop gezeigt werden, aber das Resultat hängt von der Relation zwischen den Schwingungsebenen der Polarisationsfilter und denen der doppelbrechenden Substanzen ab. Sind die Filterebenen senkrecht zueinander (gekreuzt) wird das Sehfeld dunkel und doppelbrechende Substanzen werden nur sichtbar, wenn die eigenen Schwingungsebenen nicht mit denen der Filterebenen parallel sind. Dunkel werden aber auch Gebiete, wo überhaupt keine doppelbrechende Substanzen vorkommen. Die Analyse fordert deswegen eine Drehung des Kreuztisches, was aber beim Photographieren nicht möglich ist. Es ist folglich auch unmöglich aus einer Photographie herauszufinden, ob eine dunkle Stelle im Bilde auf Parallelstellung oder auf Nichtbefinden doppelbrechender Substanzen beruht.

Im Zahnschmelz liegen die doppelbrechenden Substanzen im grossen und ganzen zu den Prismen parallel. Da aber die Prismen nicht immer gerade verlaufen, können Prismen oder Prismenteile dunkel erscheinen, obwohl sie gut mineralisiert sind. Um eine Analyse auch aus der Photographie zu erlauben, ist hier das Polarisationsmikroskop in der Weise abgeändert, dass der Analysator oderhalb des Okulars liegt und drehbar ist. Durch eine Stange wird der Analysator mit dem ebenfalls drehbaren Polarisator verbunden und beide in gekreuzter Stellung während der Exponierung gedreht. Dadurch sind doppelbrechende Teile zweimal in der Lage aufzuleuchten und werden auf der Photographie sichtbar.

Address: *School of Dentistry,*
University of Lund,
Malmö, Sweden