Scanning Microscopy

Volume 1 | Number 3 Article 29

4-6-1987

Scanning Electron Microscopy in Oral Mucosal Research: A Review

A. Kullaa-Mikkonen
University of Kuopio

Scanning Microscopy, Vol. 1, No. 3, 1987 (Pages 1145-1155) 0891-7035/87$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

SCANNING ELECTRON MICROSCOPY IN ORAL MUCOSAL RESEARCH: A REVIEW

A. Kullaa-Mikkonen

Department of Oral Surgery
Faculty of Dentistry, University of Kuopio
Finland

(Received for publication December 03, 1986, and in revised form April 06, 1987)

Abstract

This review paper highlights some aspects of the contribution of SEM in the field of oral mucosa research. These include 1) different preparative techniques, 2) structure of the oral mucosa and its role in normal function, 3) advances in oral microbiology, 4) development of the oral mucosal epithelium, 5) pathological diagnosis and 6) morphometry.

There are four main ways to study the oral mucosa with SEM; biopsy (autopsy) samples, smears, replica technique, and cell culture techniques. The structural studies can be divided as studies of the surface structure of the superficial cells of the oral mucosa and studies of the interactions between epithelium and connective tissue. Colonization and the morphology of micro-organisms are easy to see with SEM.

Morphometric techniques have been used to determine the density of connective tissue papillae and to analyse surface structures of epithelial cells. In this paper, computerized image analysis systems for use in SEM research are presented.

Key Words: Scanning electron microscopy, oral mucosa, tongue, microplicae, micro-organisms, keratinization, oral pathology, morphometry.

Address for correspondence:
Arja Kullaa-Mikkonen
Department of Oral Surgery, Faculty of Dentistry
University of Kuopio, P.O.Box 6, 70211-Kuopio
Finland Phone No. 358 71.162275

Introduction

Since its beginning, scanning electron microscopy (SEM) has become a tool used by many investigators in oral biology and oral pathology. Scanning electron microscopy was first applied in studies of the oral mucosa by Morgenroth & Morgenroth (64, 65). Since that time preparative techniques have been evaluated and greatly improved. During recent years, SEM studies of oral mucosa have accumulated in fields ranging from basic science to studies of specific mucosal problems, such as development of the oral mucosa, adherence of micro-organisms, and pathological variation.

As many studies have shown, SEM-investigations of the surface structures of oral lesions are worthwhile since they add information to the findings of light microscopy and transmission electron microscopy (TEM). With SEM it is possible to study the most superficial layers as three-dimensional pictures, and a large area of the mucosa can be studied at the same time and at high magnification. SEM may also provide an appropriate mean to test the diagnosis of certain alterations of the mucosa, including premalignant and malignant lesions. Many gaps, however, still exist in our knowledge of the SEM structure of the oral mucosa. In the following paper the findings of different SEM-studies are summarized in order to form the basis for further SEM-studies of oral mucosa and to describe the use of SEM in oral biology and oral pathology. A quantitative approach is seldom used to analyse surface structures (44-48, 52-54, 66), and SEM studies of the oral mucosa are usually descriptive. Therefore, in the last section of this review I present some possibilities for analyzing SEM-structures quantitatively and especially with a computer.

Preparative techniques for various structural studies

Preparation of a tissue specimen of oral mucosa for SEM is a complex procedure, which can introduce major topographic distortion and/or fine structural flaws. Workers should consider some aspects of their methods of specimen preparation: 1) assessing how much fine structure is disturbed by obtaining the specimen and physically handling

it, 2) preparing a surface free from coating material (e.g., mucus, blood, tissue fluid), 3) minimizing specimen curling and shrinking during fixation, dehydration and drying, 4) developing other methods of "opening up" internal organization, because the surface of a simple section does not always reveal significant information. The method that has gained wide acceptance for preparing biological specimens for SEM is: fixation with an aldehyde, postfixation with OsO_4, dehydration with critical point technique and sputtered with gold (5, 14, 51). Biopsy (not including autopsy)samples must be taken under local anesthesia, but cell samples taken with a curette can be obtained without topical anesthesia. When not injected into the biopsy area, however, topical anesthesia did not cause any tissue damage (43). For removing coating material the best results were obtained when saline solution was used to wash the specimen before fixation (48). This method is simple and rapid and does not affect the structure of the epithelial cells (48). When used to eliminate the coating material, enzymes usually destroy the fine structure of cells (14). To prevent the tissue from curling up and shrinking during fixation, the specimen was carefully fastened to a styrofoam plate with two pins (43). The shrinkage of our specimens was about 30 % (48), whereas in formalin-fixed and paraffin-embedded chicken spleen it was about 40 % (76). Smears for SEM are usually handled as a biopsy specimen. With SEM, malignant cells are easy to identify (52, 53, 75). The disadvantage of the smear technique, compared to the use of tissue specimens, is the lack of any histological continuity.

Biopsy specimens provide further possibilities to study different parameters of tissues. For example, interaction between epithelium and connective tissue can be studied when epithelium is separated from the underlying lamina propria by maceration (42, 68, 71, 80, 99) or mechanically with the use of microdissection instruments (70). Subepithelial connective tissue can also be studied in a freshly cut surface. The cell surface of different layers of epithelia can be studied when the epithelial cells are first separated from the various epithelial layers by trypsin digestion (20) stripping tape technique (32, 60), or using the freeze-fracture method (58).

For meaningful SEM studies of microbial populations associated with surfaces, it is essential that the specimens be modified as little as possible during their preparation. The composition of the fixative significantly influences the number of micro-organisms preserved. Karnovsky's fixative with Ruthenium red best preserved surface-associated organisms (28). McMillan (59) suggested, however, that all the Ruthenum red and Alcian blue positive material in close association with the exposed surface of the epithelial cells is precipitated saliva. The other important factors of specimen preparation for studying surface-associated micro-organisms are the washing of specimens prior to fixation, storage of fixed specimens, and handling and storage of critical point dried specimens.

The cellular fine structure of epithelium and connective tissue can be seen easily when SEM is used after in vitro maintenance of explants (10, 78). This in vitro system using cultured epithelial cells is useful for studying the proliferation and morphology of epithelial and connective tissue cells and epithelia - connective tissue interactions. It is also suitable for investigating the effect of various substances suspected of influencing these mechanisms.

A non-invasive method used to study the fine surface structure is the replica technique (49). This method is suitable for studying the masticatory mucosa, which overlies bone and is stiff and immobile. The disadvantages of this method, however, are the limited possibilities to study various parameters (only the surface of superficial cells) and the lack of histological control.

Structural studies of oral mucosal epithelium

The entire oral cavity is lined by stratified squamous epithelium which forms the primary structural barrier between the internal and external environments. The epithelium and the underlying connective tissue show functionally related regional variation in their structures (62, 86). Using SEM at low magnification, the surface structure of the oral mucosa is smooth and does not differ in different parts of the oral cavity (45). Only the tongue surface with its papillary structure forms a very specialized mucosa (44, 45). At high magnification the characteristic difference in the regional variation of the surface structure has been demonstrated in many studies and in many species (1-4, 18-22, 30, 38, 44, 56, 57). The surface of the superficial cells contains ridge-like surface folds with a different morphology. These surface folds are discussed under various names, such as cytoplasmic folds or micro-folds (96), microridges (41, 74, 85), microplicae (2, 19, 21, 44-48, 66), and microrugae (8). The term "microplicae" has gained wide acceptance. The microplicae of the superficial epithelial cells are characteristic of the cells of non-keratinized epithelium; e.g., epithelium of cheek, soft palate, floor of the mouth and ventral surface of the tongue (lining mucosa). Cells of keratinized epithelium, which have a pitted or honeycombed appearance, are found in epithelial cells of the hard palate and attached gingiva (masticatory mucosa). Changes in the cell surface also indicate the thickening of the plasma membrane during cell maturation. These marked regional variations in the structure of the oral mucosa are related to rates of surface wear, which are affected both by the degree of surface trauma at any particular site and by resistance of the epithelial surface to abrasion.

Differences in the structure of the mucosa reflect variations in function. To permit movement and extension, lining mucosae must be elastic and the epithelium have large cells with pleated cell walls, large amounts of intercellular glycoprotein, and elastic fibres. The

origin and functional role of microplicae on the cell surface of lining mucosa is a controversial issue. Four different hypotheses about the function of microplicae have been reviewed by Nair and Schroeder (66): 1) intercellular interdigitation for cell adhesion, 2) a protective function by reducing the surface area of contact, 3) aiding the laminar flow of surface protecting and lubricating secretions, 4) a reserve surface area for cell stretching. Microplicae are typical of the surfaces of areas covered with protective mucus, such as the cervical mucosa (77), the epidermis of the human fetus (29) and the esophageal mucosa (85). When mechanical stress is great enough, however, the cells become fully keratinized with a pitted appearance, like the cells of the masticatory mucosa. Masticatory mucosae, being required to resist physical forces yet remain immobile, have a massive and inflexible stratum corneum, a greater area of epithelial-connective tissue interface, and a collagenous lamina propria with large and straight collagen fibrils (42, 99).

The epithelial cells perform a number of specialized synthetic activities associated with the maintenance of a surface barrier. These include, for example, the synthesis of cell surface and extracellular components related to cell adhesion (95). Microplication and the pitted appearance of the cell surface are thought to be associated with the mechanical adhesion of cells (20, 99). This mechanism has been studied in different layers of epithelium (20). The adhesion and cell morphology is destroyed, for example, in inflammatory epithelium (39) or in epithelial tumors.

Cell junctions play a major role as a barrier against entry of noxious substances or organisms, and also against loss of fluids. At present, however, little is known about SEM-findings in this field, although normal cell junctions can be tight or ovelapping (3, 44-47, 56, 97). The role of the gap (seen also with TEM, 82) between two adjacent cells is poorly understood.

Clearly, the interactions between epithelium and connective tissue are of clinical significance in relation to processes such as the control of cell proliferation and migration. Studied with SEM, the epithelium - connective tissue interface can be identified as different in three regions: (1) floor of the mouth, (2) lip and cheek, (3) gingiva and hard palate (42). The floor of the mouth shows the lowest connective tissue papillae density, the smallest papillae, and connective tissue plateaux separated by narrow grooves. Lip and cheek mucosae reveal an intermediate density, the papillae are frequently bifurcated and angulated. Gingiva and hard palate are characterized by the highest papillary density and by papillae which are cylindrical, slender and erect. The alveolar mucosa exhibits intermediate features between those of the floor of the mouth and those of the cheek mucosa (42). Under several pathological conditions, the epithelium - connective tissue interactions may change, for example, in lichen planus (33), submucous fibrosis (69) and leukoplakia (34, 55).

The tongue surface with its papillary structure has been studied extensively in both humans and other species (9, 11-13, 16, 36, 37, 44, 83, 84, 91, 100, 101). SEM techniques have provided valuable information on the tongue mucosa, as well as in studies of pathological changes of the tongue (see the section "SEM in oral pathology"). The light microscopical structure of tongue epithelium can be seen more easily with SEM. Different types of tongue papillae, e.g., filiform, fungiform, foliate, vallate, are easy to identify with SEM. At high magnification, the structure and localization of taste pores can also be studied (6, 37, 44, 46).

Development of oral mucosa

Except for the development of the tongue papillae (9, 24, 25, 31), little SEM work on embryonic morphogenesis has been undertaken on the general structure of the oral mucosa (92, 96). Using SEM it is easier to trace the appearance and development of tongue papillae in human embyros and fetuses than with light microscopy, because the changes can be recognised earlier. Hersch and Ganchrow (31) and Dourov et al. (25) found the first signs of circumvallate papillae as early as the 8th - 12th week of embryonic development and signs of foliate papillae at about 10 weeks. Fungiform papillae begin to develop before filiform papillae, which appear at 10 - 18 weeks. In addition, in humans the surfaces of the epithelial cells lining the developing lingual epithelium exhibit characteristic changes in microplicae. According to Takagi et al. (92), the varied forms of microplicae at different developmental stages can be classified into five types, i.e., Type I microvilli, Type II short straight microplicae, Type III curved microplicae, Type IV branched microplicae, and Type V cells with a pitted appearance. In our laboratory this classification has been found in the gingival epithelium during would healing after tooth extraction (Fig.1) and analyzed with a computer (see the section "Morphometry in SEM research").

Adherence of micro-organisms to the oral mucosa

A common function of the oral epithelia of all regions is to form a relatively effective barrier to penetration of micro-organisms. Even so, a possibly significant function of the turnover of oral epithelia is rapid replacement of the epithelial surface to provide a self-cleansing mechanism which prevents undue colonization or penetration of the epithelial surface by bacteria and fungae (7, 15, 24, 35, 40, 50, 61, 79).

Seen with SEM, colonization of micro-organisms also shows regional variation. The microbial colonization has been shown to correlate with the degree of keratinization on the baboon tongue (7). Normally, the hairs of the filiform papillae of the human tongue contain a massive plaque of micro-organisms (44), whereas other healthy mucosal surfaces are usually free from micro-organisms (45).

The morphology and also the quantitative morphology of oral micro-organisms are easy to

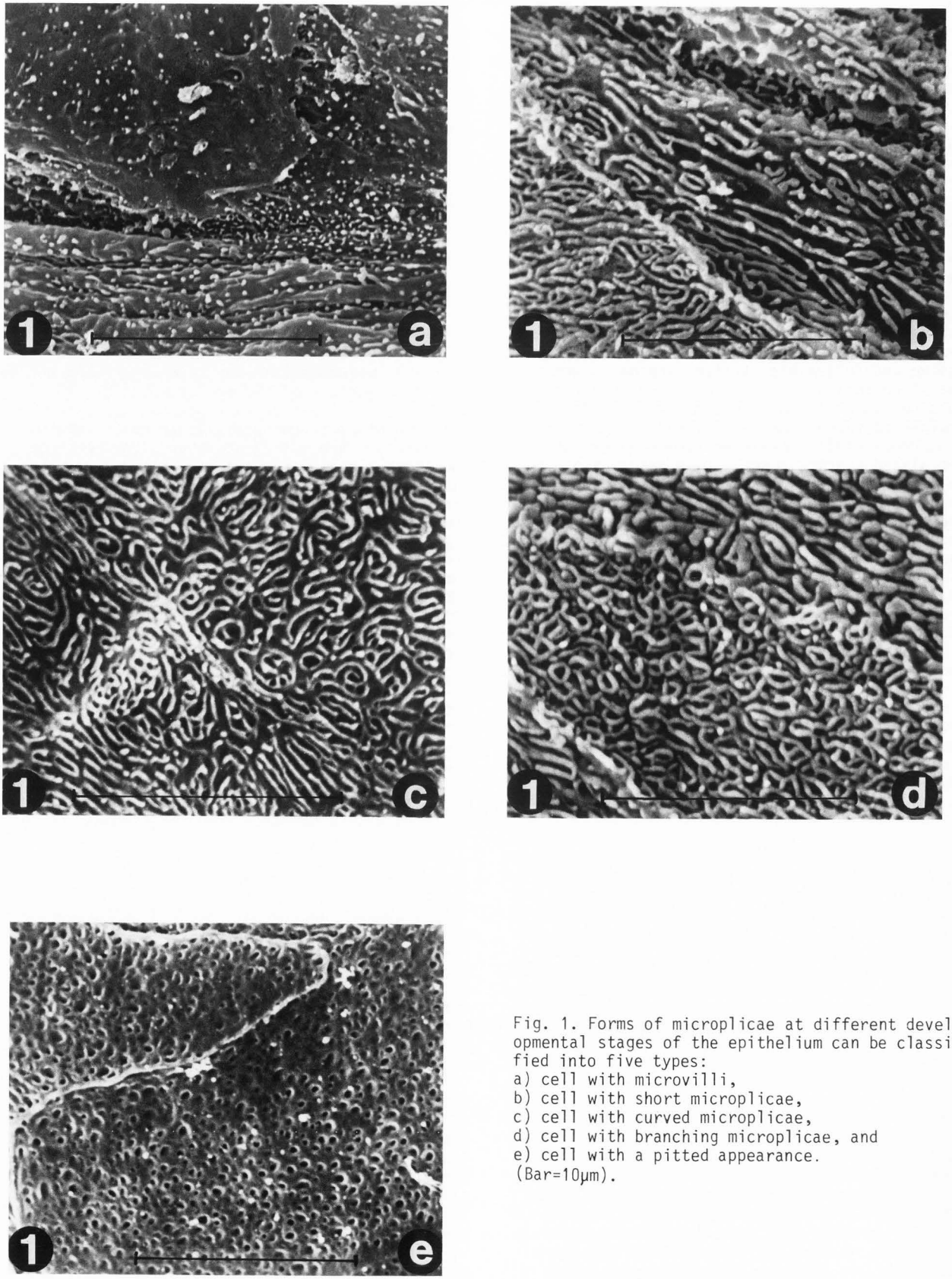


Fig. 1. Forms of microplicae at different developmental stages of the epithelium can be classified into five types:
a) cell with microvilli,
b) cell with short microplicae,
c) cell with curved microplicae,
d) cell with branching microplicae, and
e) cell with a pitted appearance.
(Bar=10μm).

elucidate. SEM-studies, for example about a multicelluar filamentous bacteria Simonsiella (Fig. 2) are very informative (23, 27).

SEM in oral pathology

SEM investigation is most suitable for studying the processes that affect the upper part of the mucous membrane. A number of generalized and localized disorders are known to affect the oral mucosa, causing changes in its keratinization and thus differences in its appearance. Lichen planus and lupus erythematosus are the most important of the generalized disorders; and leukoplakia, white spongy nevus, leukoedema, cheek biting and tobacco-induced hyperkeratoses are examples of the localized disorders. Using SEM technique, changes in the pattern of epithelial cells and in cell junctions can be seen in these pathological conditions (8, 64, 65, 70-74).

Much of the recent interest in SEM research on oral pathological conditions has focused on the tongue (87-91). The presence or absence of hairs and hairlike processes of filiform papillae can be studied more efficiently with SEM. Further changes in the structure of taste pores can also be detected using this method (46, 47). Atrophy or absence of filiform papillae can easily be seen with SEM. For example, geographic tongue with papillary bodies (Fig. 3) can be distinguished from atrophic tongue, which is characterized by pronounced flattening of the mucosal surface (Fig. 4). Many tumors of the oral mucosa, such as papillomas and fibromas, also show few surface changes (26, 71). With SEM preneoplastic cells of the cervix can be distinguished from normal epithelial cells (41, 77, 81, 98), and this method may also be valid in the early diagnosis of premalignant lesions in the oral mucosa (17, 64, 65, 67). The oral mucosa is quite similar to other mucous membranes in the human body. In addition, the new morphometric and surface labelling methods may provide more information about pathological conditions of the oral mucosa.

Morphometry in SEM research

Several SEM studies have been made about the surface features of the oral mucosa, but a quantitative approach has rarely been used to analyse these structures (44-48, 52-54, 66). Although morphometric methods have long been used in light microscopy (93) and in electron microscopic cytology (94), SEM findings are generally descriptive. Matravers and Tyldesley (52, 53) used a quantitative approach to analyse surface structures in smears of normal oral mucous membrane and squamous cell carcinomas. Morphometric techniques have been used by Klein-Szanto and Schroeder (42) to determine the density of connective tissue papillae at six different sites of the oral mucosa. Variation and density of microplication in superficial cells of normal lining mucosa have been described by Nair and Schroeder (66). Measured manually using a double lattice test system (93), the total length of microplicae ranges from 130 to 550 μm per 100 μm^2 cell surface area. The width of microplicae has been reported as 0.17 μm but can range from 0.1 μm to 0.23 μm in cells of the oral mucosa (2, 44, 45, 66). Microplicae are of the same order of magnitude as the intercellular interdigitations associated with the desmosomes observed by TEM (20). The morphometry of tongue papillae and microplicae has given new information about the normal structure of the human tongue but also of changed structure, where the papillae have been changed (43, 44, 46, 47).

In the last ten years numerous applications have been found for computerized image analysis. To my knowledge, in the field of SEM, no papers have been published about fully automatic image analysis by computer and only preliminary findings of our computer system (IBAS) can be presented. A schematic view of the computer used for fully automatic image analysis is illustrated in Figure 5. SEM micrographs of the cell surface are obtained at two levels of magnification. Computer analysis of these images is based on digitization of the picture into a set of discrete picture elements (pixels). In the computer memory each pixel is located in 2-dimentional space and characterized by a grey value. With the grey level histogram, the relevant objects can be extracted from an image. In SEM pictures, the microplicae of a cell can easily be seen (Fig. 6), and the computer can also calculate, for example, the area density of the microplicae. A recent trend is to develop accessible program packages for further studying the SEM-images. Fully automatic image analysis by computer is desirable for many reasons, among these are the high levels of invariance and reproducibility.

General summary

Although much has been done within the last ten years to clarify the SEM structure of the oral mucosa, some basic areas for further investigation can clearly be identified. For example, little is known about the adhesion of the epithelial cells and their disturbance by disease processes or about the role of the epithelial cell surface in normal function or in pathological processes. Furthermore, cell surface makers and labelling techniques which have been adapted for use with the SEM (63) can provide new information on the distribution and dynamics of specific membrane component on cell surfaces.

It is increasingly apparent that successful progress in more detailed morphometric studies would follow the use of computed morphometry. The computing system described offers a method that, combined with biochemical, histological, histochemical, autoradiographic or transmission electron microscopic investigations, is a tool in oral mucosa research. This method allows the handling of enormous amounts of raw data, transformation to morphometric parameters and the statistical treatment, which are all too time - consuming and expensive without a computer. Mucosal SEM research has not yet widely adopted such as new methods for its own purpose, but in the next few years we may expect to obtain infor-

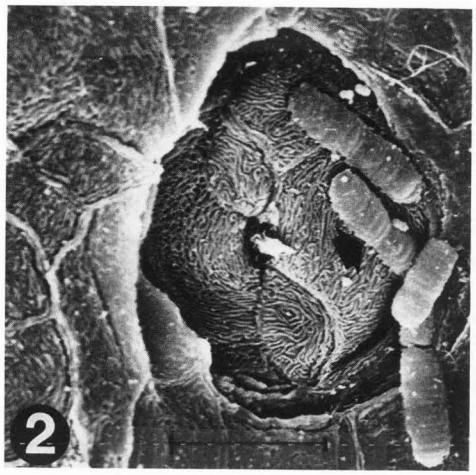
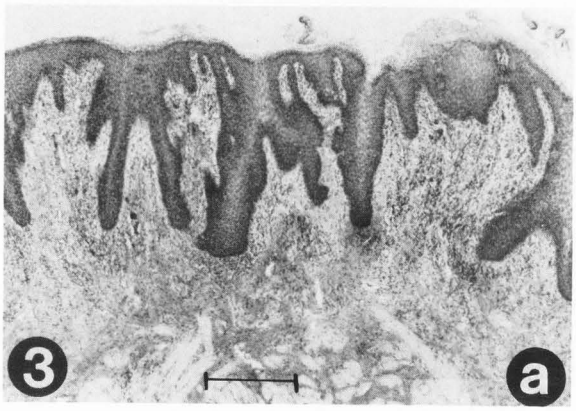
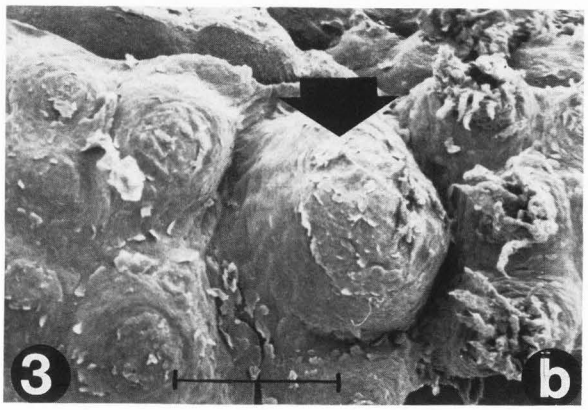


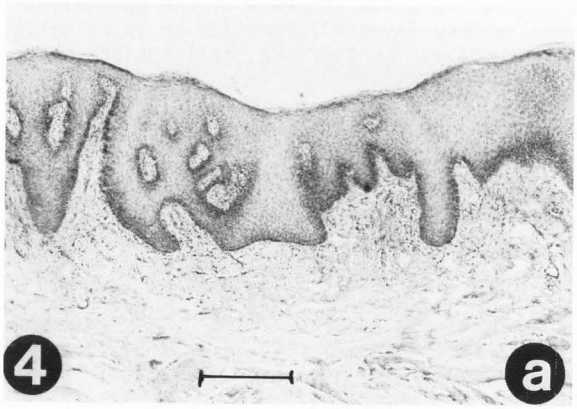
Fig. 2. Scanning electron photomicrograph of a fungiform papillae of pig tongue shows a flat, ribbon-shaped filament of Simonsiella (arrow). The segmented, multicellular morphology is clearly evident.(Bar=10 μm).

Fig. 3.
a) In geographic tongue, filiform papillae are not visible in the light microscopy. The epithelium forms long rete pegs, and inflammatory infiltration is moderate.(Bar=100 μm).
b) Surface of geographic tongue contains the bodies of filiform papillae. In the middle of the picture a fungiform papilla is visible (arrow).(Bar=1 mm).

Fig. 4.
a) In atrophic tongue the epithelium is thin and no filiform papillae are visible.(Bar=100 μm).
b) The surface of the tongue with filiform atrophy is rather smooth with low elevations. Some hairs, which are short and narrow are visible (arrows).(Bar=1 mm).







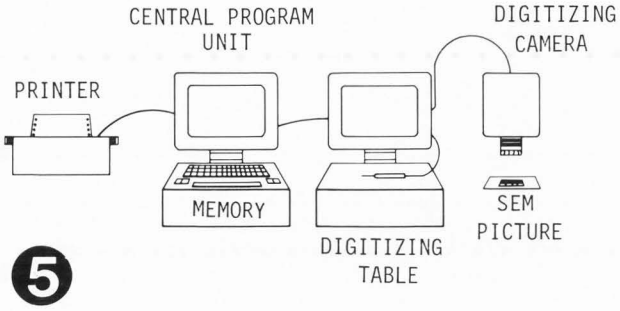
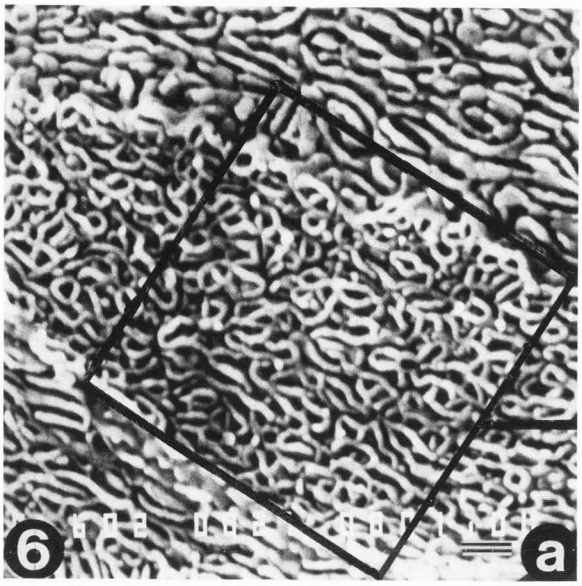
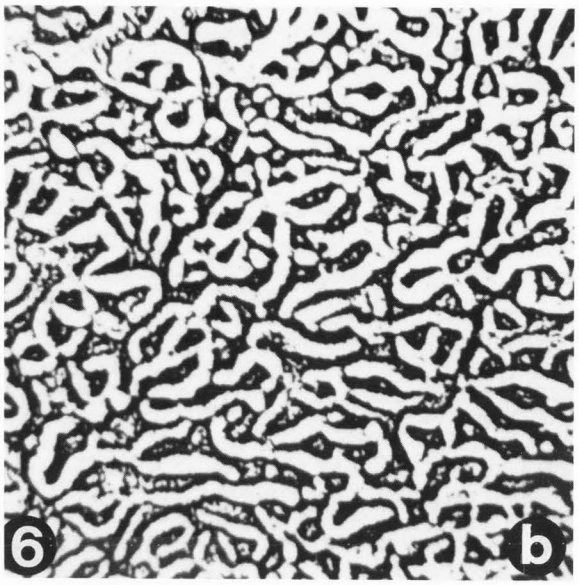


Fig. 5. Schematic representation of the computer system used for fully automatic image analysis.

Fig. 6.
a) Scanning electron photomicrograph of a epithelial cell, which has microplicae. (Bar = 1 μm).
b) A picture of the epithelial cell seen above when processed with a Laplacian highpass filter.
c) A picture operated with a boolean operation AND. This operation takes away the pixels that do not belong to the objects but are found by the Laplacian filter.
d) A picture operated with the boolean OR, which fills the holes in the objects.





mation of great interest and application to studies of the SEM structure of the oral mucosa and their underlying relevance to oral diseases.

References

1. Albrigo S, Delre G, Lovato G, Vaselli S. (1982). La mucosa gengivale normale osservata al SEM. Minerva Stomatologica 31, 427-429.
2. Andrews PM. (1976). Microplicae: Characteristic ridge-like folds of the plasmalemma. J. Cell Biol. 68, 420-429.
3. Appleton J, Tyldesley WR. (1971). Observation on the ultrastructure of the buccal epithelium of the rat. Arch. Oral Biol. 16, 1071-1088.
4. Appleton J, Heaney TG. (1977). A scanning electron microscope study of the surface features of porcine Oral mucosa. J. Periodontal Res. 12, 430-435.
5. Arenberg IK, Marowitz WE, McKenzie AP. (1971). Preparative techniques for the study of soft biological tissues in the scanning electron microscope. Trans. Am. Acad. Opthalmol. and Otolaryngol. 75, 1332-1345.
6. Arvidson K. (1976). Scanning electron microscopy of fungiform papillae on the tongue of man and monkey. Acta Otolaryngol 81, 496-502.
7. Aufdemorte TB, Cameron IL. (1981). The relation of keratinization to bacterial colonization on the baboon tongue as demonstrated by scanning electron microscopy. J. Dent. Res. 60, 1008-1014.
8. Banoczy J, Lapis K, Albrecht M. (1980). Scanning electron microscopic study of oral leukoplakia. J. Oral Path. 9, 145-154.
9. Baratz RS, Farbman AI. (1975). Morphogenesis of rat lingual filiform papillae. Am. J. Anat. 143, 283-302.
10. Bergenholz A, Hallmans G, Hanström L. (1977). Scanning electron microscopy of palatal mucosa maintained in organ culture: a method for three-dimensional visualization of cell morphology. Anat. Rec. 189, 433-442.
11. Boshell JL, Wilborn WH, Singh BB. (1979). Surface morphology and bacterial flora of the dorsum of the pig tongue. Scanning Electron Microsc. 1979; III: 363-368.
12. Boshell JL, Wilborn WH, Singh BB. (1980). A correlative light microscopic transmission and scanning electron microscopic study of the dorsum of human tongue. Scanning Electron Microsc. 1980; III: 505-510.
13. Boshell JL, Wildborn WH, Singh BB. (1982). Filiform papillae of cat tongue. Acta Anat. 114, 97-105.
14. Boyde A, Wood C. (1969). Preparation of animal tissues for surface scanning electron microscopy. J. Microscopy 90, 221-249.
15. Brady JM, Gray WA, Lara-Garcia W. (1975). Localization of bacteria on the rat tongue with scanning and transmission electron microscopy. J. Dent. Res. 54, 777-782.
16. Chamorro CA, de Paz P, Sandoval J, Fernandez JG. (1986). Comparative scanning electron-microscopic study of the lingual papillae in two species of domestic mammals (Equus caballus and bos taurus). Acta Anat. 125, 83-87.
17. Chomette G, Leclerc JP, Szpirglas H, Auriol M, Vaillant JM. (1981). Scanning electron microscopy of normal, malignant and post radiotherapeutic oral mucosal cells. Path. Res. Pract. 171, 345-352.
18. Cleaton-Jones P. (1972). Surface ultrastructure of the mucosa of the soft palate in the Vervet monkey. S. Afr. J. Med. Sci. 37, 101-104.
19. Cleaton-Jones P, Fleisch L. (1973). A comparative study of the surface of keratinized and non-keratinized oral epithelia. J. Periodont. Res. 8, 366-370.
20. Cleaton-Jones P. (1975). Surface characteristics of cells from different layers of keratinized and non-keratinized oral epithelia. J. Periodontal. Res. 10, 79-87.
21. Cleaton-Jones P. (1976). An ultrastructural study of keratinized epithelia in the rat soft palate. J. Anat. 122, 23-29.
22. Cleaton-Jones P, Buskin SA, Volchansky A. (1978). Surface ultrastructure of human gingiva. J. Periodontal Res. 13, 367-371.
23. Closset M, Dourov N, Menu R, Kaeckenbeeck A. (1985). Simonsiella, bacterie geante: Hôte buccal quelque peu meconnu. Etude microbiologique et ultrastructurale. Bull. Group. int. Rech. Stomat. Odont. 28, 163-176.
24. Dourov N, Coremans-Pelseneer J. (1980). Etude en microscopie électonique à balayage de la candidose linguale expérimentale chez le rat. Jour. Biol. Buccale 8, 161-173.
25. Dourov N, Milaire J, Arys A. (1981). Etude en microscopie électronique à balayage de la surface de la muqueuse linguale de l'embryon et du foetus humains. Bull. Group. int. Rech. sc. Stomat. et Odont. 24, 219-233.
26. Dourov N. (1984). Scanning electron microscopy contribution in oral pathology. Scanning Electron Microsc. 1984; I: 243-248.
27. Garandina G, Bacchelli M, Virgili A, Strumia R. (1984). Simonsiella Filaments Isolated from Erosive Lesions of the Human Oral Cavity. J. Clin. Microbiol. 19, 931-933.
28. Garland CD, Lee A, Dickson MR. (1979). The preservation of surface associated micro-organisms prepared for scanning electron microscopy. J. Microscopy 116, 227-242.
29. Halbrook KA, Odland GF. (1975). The fine structure of developing human epidermis: light, scanning, and transmission electron microscopy of the periderm. J. Invest. Dermat. 65, 16-38.
30. Hayward AF, Hamilton AI, Hackermann MMA. (1973). Histological and ultrastructural observation on the keratinizing epithelia of the rat. Archs. Oral Biol. 18, 1041-1057.
31. Hersch M, Ganchrow D. (1980). Scanning electron microscopy of developing papillae on the tongue of human embryos and fetuses. Chemical Senses 5, 331-341.
32. Hodgkins JFW, Watkins R, Walker DM. (1978). Correlated scanning and transmission electron microscopy of cell surfaces at various levels in human gingival epithelium. Archs Oral Biol. 23, 355-360.
33. Holmstrup P, Dabelsteen E. (1979). Changes in carbohydrate expression of lichen planus - affected epithelial cell membranes. J. Invest Dermatol. 73, 364-367.

34. Holmstrup P, Dabelsteen E, Roed-Petersen B. (1981). Oral leukoplakia transplanted to nude mice. Scand. J. Dent. Res. 89, 19-26.
35. Howlett JA. (1976). The infection of rat tongue mucosa in vitro with five species of candida. J. Med. Microbiol. 9, 309-315.
36. Iida, M, Yoshioka I, Muto H. (1985). Three-dimensional and surface structures of rat filiform papillae. Acta Anat. 121, 237-244.
37. Jasinski A. (1979). Light and Scanning Microscopy of the Tongue and its Gustatory Organs in the Common Toad, Bufo bufo (L.). Z. mikrosk.-anat. Forsch. (Leipzig) 93, 465-476.
38. Kaplan BG, Pameijer CH, Ruben MP. (1977). Scanning electron microscopy of sulcular and junctional epithelia correlated with histology (Part I). J. Periodontol. 48, 446-451.
39. Kaplan GB, Ruben MP, Pameijer CH. (1977). Scanning electron microscopy of the epithelium of the periodontal pocket - Part II. J. Periodontol. 48, 634-638.
40. Karring T, Löe H. (1970). The three-dimensional concept of the epithelium-connective tissue boundary of gingiva. Acta Odont. Scand. 28, 917-933.
41. Kenemans P, Davina JHM, de Haan RW, ven der Zanden P, Vooys GP, Stolk JG, Stadhouders AM. (1981). Cell surface morphology in epithelial malignancy and its precursor lesions. Scanning Electron Microsc. 1981; III: 23-36.
42. Klein-Szanto AJP, Schroeder HE. (1977). Architecture and density of the connective tissue papillae of the human oral mucosa. J. Anat. 123, 93-109.
43. Kullaa-Mikkonen A, Sorvari TE, Kotilainen R. (1985). Morphological variations on the dorsal surface of the human tongue. Proc. Finn. Dent. Soc. 81, 104-110.
44. Kullaa-Mikkonen A, Sorvari TE. (1985). A scanning electron microscopic study of the dorsal surface of the human tongue. Acta Anatomica. 123, 114-120.
45. Kullaa-Mikkonen A. (1986). Scanning electron microscopic study of surface of human oral mucosa. Scand. J. Dent. Res. 94, 50-56.
46. Kullaa-Mikkonen A. (1986). Geographic tongue: an SEM study. J. Cut. Pathol. 13, 154-162.
47. Kullaa-Mikkonen A, Sorvari TE. (1986). A scanning electron microscopic study of fissured tongue. J. Oral Pathol. 15, 93-97.
48. Kullaa-Mikkonen A. (1987). Plication density of epithelial cell surface of oral mucosa. An evaluation of various methods of preparation and mathematical background to morphometry. (In press).
49. Lilienthal B. (1977). In vivo replica techniques for SEM. J. Dent. Res. 56, 444.
50. Malcom SA, Hughes TC. (1980). The demonstration of bacteria on and within the stratum corneum using scanning electron microscopy. Br. J. Dermat. 102, 267-275.
51. Marovitz WF, Arenberg IK, Thalmann R. (1970). Evaluation of preparative techniques for the scanning electron microscope. Laryngoscope 80, 1680-1700.
52. Matravers J, Tyldesley WR. (1978). Scanning electron microscopy of oral epithelial cells. Part I. Normal and malignant tissue. Brit. J. Oral Surg. 15, 193-202.
53. Matravers J, Tyldesley WR. (1978). Scanning electron microscopy of oral epithelial cells. Part II. Potentially malignant lesions (A computer-assisted study). Br. J. Oral Surg. 15, 203-214.
54. Matravers JM, Heaney TG, Appleton J. (1982). Computer analysis of the surface ultrastructural features of porcine oral mucosa. Archs. Oral Biol. 27, 481-485.
55. McKenzie IC, Dabelsteen E, Roed-Petersen B. (1979). A method for studying epithelial-mesenchymal interactions in human oral mucosal lesions. Scand. J. Dent. Res. 87, 234-243.
56. McMillan MD. (1974). A scanning electron microscopic study of the keratinized epithelium of the hard palate of the rat. Archs. Oral Biol. 19, 225-229.
57. McMillan MD. (1979). The surface structure of the completely and incompletely orthokeratinized oral epithelium in the rat: A light, scanning and transmission electron microscope study. Am. J. Anat. 156, 337-352.
58. McMillan MD. (1979). The complementary structure of the superficial and deep surfaces of the stratum corneum of the hard palate in the rat. J. Periodontal Res. 14, 492-502.
59. McMillan MD. (1980). Transmission and scanning electron microscope studies on the surface coat of the oral mucosa in the rat. J. Periodontal Res. 15, 288-296.
60. McMillan MD, Smillie AC, Gray DW. (1982). The surface structure of the epithelium of the hamster cheek pouch. Archs. Oral Biol. 27, 623-634.
61. Merrell BR, Walker RI, Joseph SW. (1984). In vitro and in vivo pathologic effects of Vibrio parahaemolyticus on human epithelial cells. Can. J. Microbiol. 30, 381-388.
62. Meyer J, Medak H. (1962). Keratinization of the oral mucosa. In: Fundamentals of Keratinization. EO Butcher, RF Sognnaes, (eds.). American Association for the Advancement of Science, Washington, D.C. pp. 139-149.
63. Molday RS, Maher P. (1980). A review of cell surface makers and labelling techniques for scanning electron microscopy. Histochem. J. 12, 273-315.
64. Morgenroth K, Morgenroth Jr, K. (1970). Vergleichende stomatoskopische und rasterelektronenmikroskopische Untersuchhungen von Mundschleimhautveränderungen. Dtsch. zahnärzfl. Z. 25, 199-207.
65. Morgenroth K, Morgenroth Jr, K. (1970). Rasterelektronenmikroskopische Untersuchungen zur Morphologie der Leukoplakie der Mundhöhle. Dtsch. zahnärzfl. Z. 25, 1054-1060.
66. Nair PNR, Schroeder HE. (1981). Variation and density of microplications in superficial cells of the normal oral lining mucosa in the monkey Macacus fascicularis. Archs Oral Biol. 26, 837-843.
67. Nakao I. (1983). Comparative studies of exfoliated cells from the oral mucosa by light and scanning electron microscopy. Tsurumi Shigaku 9, 151-177.
68. Ooya K, Tooya Y. (1981). Scanning electron microscopy of the epithelium-connective tissue interface in human gingiva. J. Periodontal Res. 16, 135-139.

69. Pindborg JJ. (1980). Incidence and early forms of oral submucous fibrosis. Oral Surg. 50, 40-44.
70. Regezi JA, Deegan MJ, Hayward JR. (1978). Lichen planus: Immunologic and morphologic identification of the submucosal infiltrate. Oral Surg. 46, 44-52.
71. Reichart PA, Althoff J. (1979). Study of Biopsies from Lesions of the Oral Mucosa by Scanning Electron Microscopy (S.E.M.). J. Max.-fac. Surg. 7, 218-224.
72. Reichhart PA, Althoff J. (1982). Granular type of denture stomatitis. A scanning electron microscopic study of epithelial surface patterns. Oral Surg. 54, 66-72.
73. Reichart PA, Althoff J. (1983). Oral leukoplakia: a scanning electron microscopic study of epithelial surface patterns. Int. J. Oral Surg. 12, 159-164.
74. Reichart P, Böning W, Srisuwan S, Theetranont C, Mohr U. (1984). Ultrastructural findings in the oral mucosa of betel chewers. J. Oral Pathol. 13, 166-171.
75. Richter W. (1982). Die Stereomikroskopie exfoliierter Epithelzellen. Arch Otorhinolaryngol. 236, 185-195.
76. Romppanen T. (1981). Morphometry of chicken spleen germinal centers-influence of "growing" of reference volume on interpretation of stereological parameters. Proc 3rd Eur Symp Stereol, Stereol Iugosl 3 (Suppl 1), 429-434.
77. Rubio CA, Kranz I. (1976). The exfoliating cervical epithelial surface in dysplasia, carcinoma in situ and invasive squamous carcinoma. Acta Cytol. 20, 144-150.
78. Saglie R, Johansen JR, Tollefsen T. (1975). Scanning electron microscopic study of human gingival epithelial cells on the surface of teeth and glass slides. J. Periodontal Res. 10, 191-196.
79. Saglie R, Carranza Jr, FA, Newman MG, Pattison GA. (1982). Scanning electron microscopy of the gingival wall of deep periodontal pockets in humans. J. Periodontal Res. 17, 284-293.
80. Schenk P, Wersäl J. (1977). Die Ultrastruktur der Papillae filiformes der menschlichen Zunge. Arch. Derm. Forsch. pp. 1-26.
81. Sherman AI. (1977). Comparison of cancer cell surfaces of the lower reproductive tract by scanning electron microscopy. Am. J. Obstet. Gynecol. 129, 893-908.
82. Shimono M, Clementi F. (1976). Intercellular Junctions of Oral Epithelium. I. Studies with Freeze-Fracture and Tracing Methods of Normal Rat Keratinized Oral Epithelium. J. Ultrastructure Res. 56, 121-136.
83. Singh BB, Boshell JL, Steflik DE, McKinney Jr, RV. (1980). A correlative light microscopic, scanning and transmission electron microscopic study of the dog tongue filiform papillae. Scanning Electron Microsc. 1980; III: 511-516.
84. Skach M, Svedja J. (1972). Die fadenförmigen Papillen der menschlichen Zunge im Raster-Electronenmikroskop (Scanning electron microscope). Dtsch. Zahn. Mund. Kieferheilk 58, 2-9.
85. Sperry DG, Wassersug RJ. (1976). A Proposed Function of Microridges on Epithelial Cells. Anat. Rec. 185, 253-258.
86. Squier CA, Johnson NW, Hackemann M. (1975). Structure and function of normal human oral mucosa. In: Oral Mucosa in Health and Disease. (Ed). Dolby AE. Blackwell Scientific Publications, Oxford. pp.1-95.
87. Svedja J, Skach M. (1971). Die Zunge der Ratte im Raster-Elektronen mikroskop (stereoscan). Z. Mikrosk. Anat. Forsch. 84, 101-116.
88. Svedja J, Janota M. (1974). Scanning electron microscopy of the papillae foliate of the human tongue. Oral Surg. 37, 208-216.
89. Svedja J, Skach M. (1975). The three - dimensional image of lingual papillae. Folia Morphol. (Praha) 23, 145-149.
90. Svedja J, Skach M, Plackova A. (1977). Hairlike variations of filiform papillae in the human tongue. Oral Surg. 43, 97-105.
91. Svedja J, Skach M, Vaskova J. (1980). Die fadenförmigen Papillen der Zunge in Rasterelektronenmicroskop bei Menschen mit kongenitalen Herzanomalien. Zahn, Mund, Kieferheilkd. 68, 43-53.
92. Takagi T, Saito H, Aso N. (1976). Mechanism on the differentiation of microridges. Scanning electron microscopy of the surface structures of epithelial cells of the developing human tongue (in Japanese). Jap. J. Oral Biol. 18, 418-434.
93. Weibel ER, Elias H (eds.) (1967). Quantitative methods in morphology. Springer-Verlag, Berlin. pp.1-68.
94. Weibel ER (1969). Stereological principles for morphometry in electron microscopic cytology. Int. Rev. Cytol. 26, 235-302.
95. Weinmann JP, Meyer J, Medak H. (1960). Correlated differences in granular and keratinous layers in the oral mucosa of the mouse. J. Invest. Dermatol. 34, 423-431.
96. Whittaker DK, Adams D. (1971). The surface layer of human foetal skin and oral mucosa: A study by scanning and transmission electron microscopy. J. Anat. 108, 453-464.
97. Wilding RJC. (1973). The surface ultrastructure of normal, denture-bearing and denture stomatitis mucosa. J. Dent. Assn. S. Afr. 28, 576-581.
98. Williams AE, Jordan JA, Allen JM, Murphy JF. (1973). The Surface Ultrastructure of Normal and Metaplastic Cervical Epithelia and of Carcinoma in Situ. Cancer Res. 33, 503-513.
99. Wysoki GP, Wallitschek J, Hardie J. (1978). Epithelium - connective tissue interface of oral mucous membranes. Oral Surg. 45, 416-423.
100. Yoshioka I, Muto H. (1976). Surface structure of the tongue, palate and buccal mucosa of the rat. Okajimas Fol. Anat. Jap. 52, 297-312.
101. Yoshioka I, Ooishi M, Muto H. (1977). Spatial Distribution and Arrangement of Mouse Fungiform Papillae (Scanning Electron Microscopic Studies on the Oral Mucosa 4). Okajimas Fol. Anat. Jap. 54, 61-74.

Discussion with Reviewers

K. Arvidson: Under "Preparative techniques.." nothing is mentioned about using autopsy material. Do you have any experience of using material "immediately" after death and what are the risks for artefacts?

Author: The methods presented in the text are also suitable for autopsy material. Personally I do not have any experience in using material "immediately" after death, since it is impossible to get the autopsy material rapidly enough into our SEM-laboratory. Moreover, many diseases causing death, such as cardiovascular diseases, also cause changes in the surface structure of the tongue.

K. Arvidson: To remove coating materials, have you tried 0.1 M $CaCl_2$?

Author: Yes, but I saw that the results were virtually the same. Saline solution is better for our study, as we make other studies (such as immunological studies) on the other half of the same specimen, and therefore the 0.1 M $CaCl_2$ solution is not suitable.

B. Forslind: The microfolds or plicae on the surface of mucosal cells may to a certain extent be preparation artifacts. Have any studies been undertaken to establish the effect of the fixation on the surface topography of the mucosal cells?

Author: There are some studies (reference in the text) in which the effect of the fixation has been studied. I myself have studied the differences between four fixatives and have found the neutral formalin did not change the morphology of the cell surface. Furthermore, the size of the microplicae is about the same as the intercellular interdigitations associated with the desmosomes as seen in TEM.

B. Forslind: Concerning non-invasive methods to study the surface fine structure by replication would you comment how to do it in practice and what type of replication material is suitable for a wet cellular surface in vivo?

Author: The low viscosity silicone-base impression material (Xantopren Light Body, BAYER, West Germany) is a good replication material. On the impression an epoxy resin (Spurr epoxy) is cast in order to produce positive replicas, which are coated with gold.

N. Dourov: Do you find functional or/and topographical differences between cells with microridges (or "microplicae") in a regular parallel arrangement and cells with circonvoluted microridges?

Author: Yes, the degree of keratinization correlated with the type of microplicae so that the circonvoluted thick microridges were typical in the epithelium of orthokeratosis when studied in different leukoplakias. Thin parallel arrangement of the microplicae was found in the cell without keratin.

My intention is now to study the topographical differences between cells of different microplication patterns with the automatic image analysis method presented in this paper.