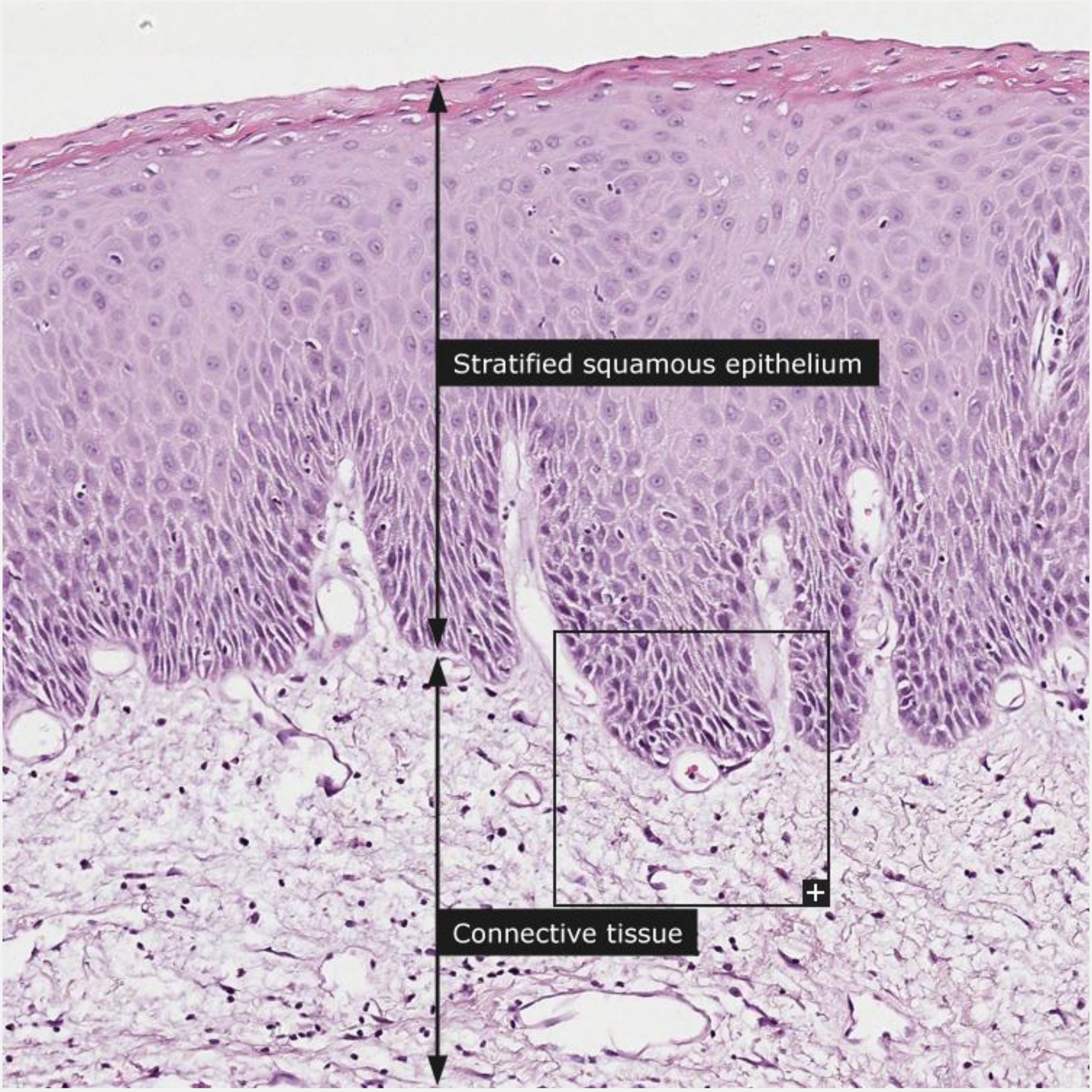
A CLINICAL TIP (1-20) OFFERED BY

PIERREL



Fluorescence and the oral cavity

Understanding the biological mechanisms underlying the fluorescence of tissues inside the mouth

A. The layering of the mucous membrane of the oral cavity

The oral mucosa is essentially composed of a stratified squamous epithelium (surface side) overlying a connective tissue (called stroma or lamina propria) from which it is separated through a basement membrane. Collagen, blood capillaries and lymphatic capillaries are in the stroma. At the base of the stroma, there is a sub-mucosa. Keratin is also present in the mucosa.

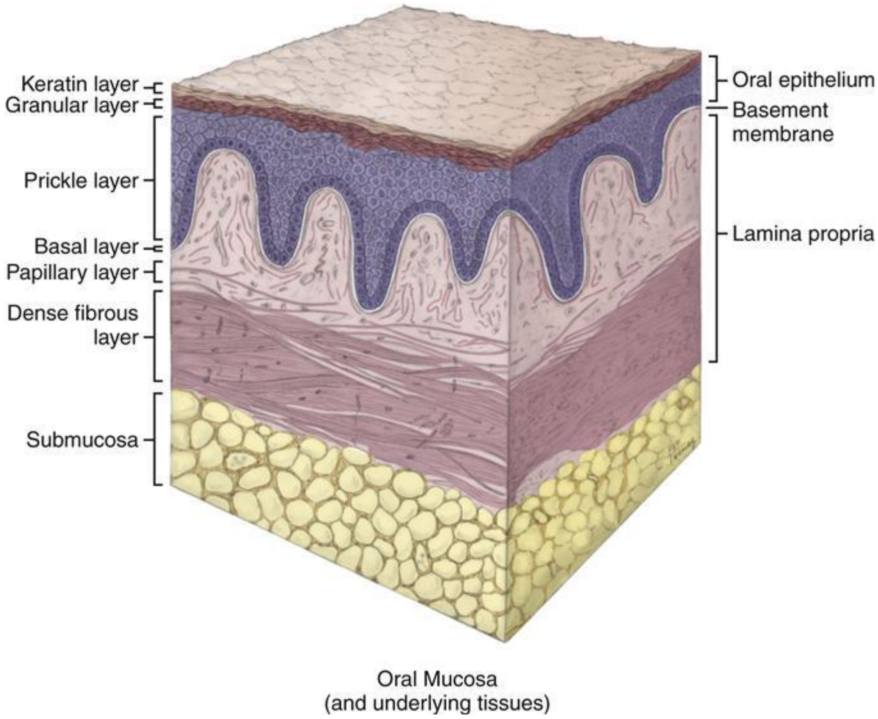


Fig.1

When we illuminate the oral cavity with a normal white light (simplistically composed of blue, green, yellow and red light) we see, as for all objects, the phenomenon of light diffusion or diffuse reflection.

In the oral mucosa, the shorter-wavelength light rays (blue light photons) are absorbed more than the longer wavelength light rays (red light photons) which return to the surface and appear inside the mouth as red or pink. The color that we see is therefore entirely reflected by the mucosa since the mouth does not naturally generate any photons.

The light with short wavelength (blue light) penetrating the mucosa, causes the reaction of the natural fluorophores present in the cells of the layered epithelium and the stroma.

The fluorophores are atoms or molecules capable of absorbing light energy in a particular spectrum of wavelength, with a consequent increase of the energy level of the electrons and their subsequent release of energy in the form of light at a longer wavelength than that absorbed, which allows the electrons to return to the initial energy state of greater stability.

These fluorophores, therefore, react to blue light by absorbing it and emitting light with a longer wavelength of green, yellow, red colour.

Therefore, using a blue light source, it is possible to provoke the reaction of the fluorophores of the mouth, visible due to particular optical filters such as those in the Goccles eyewear. These optical filters cut or filter the photons of light of the other wavelengths by isolating only the fluorescent light response green from healthy cell fluorophores, thus making the oral mucosa look green.

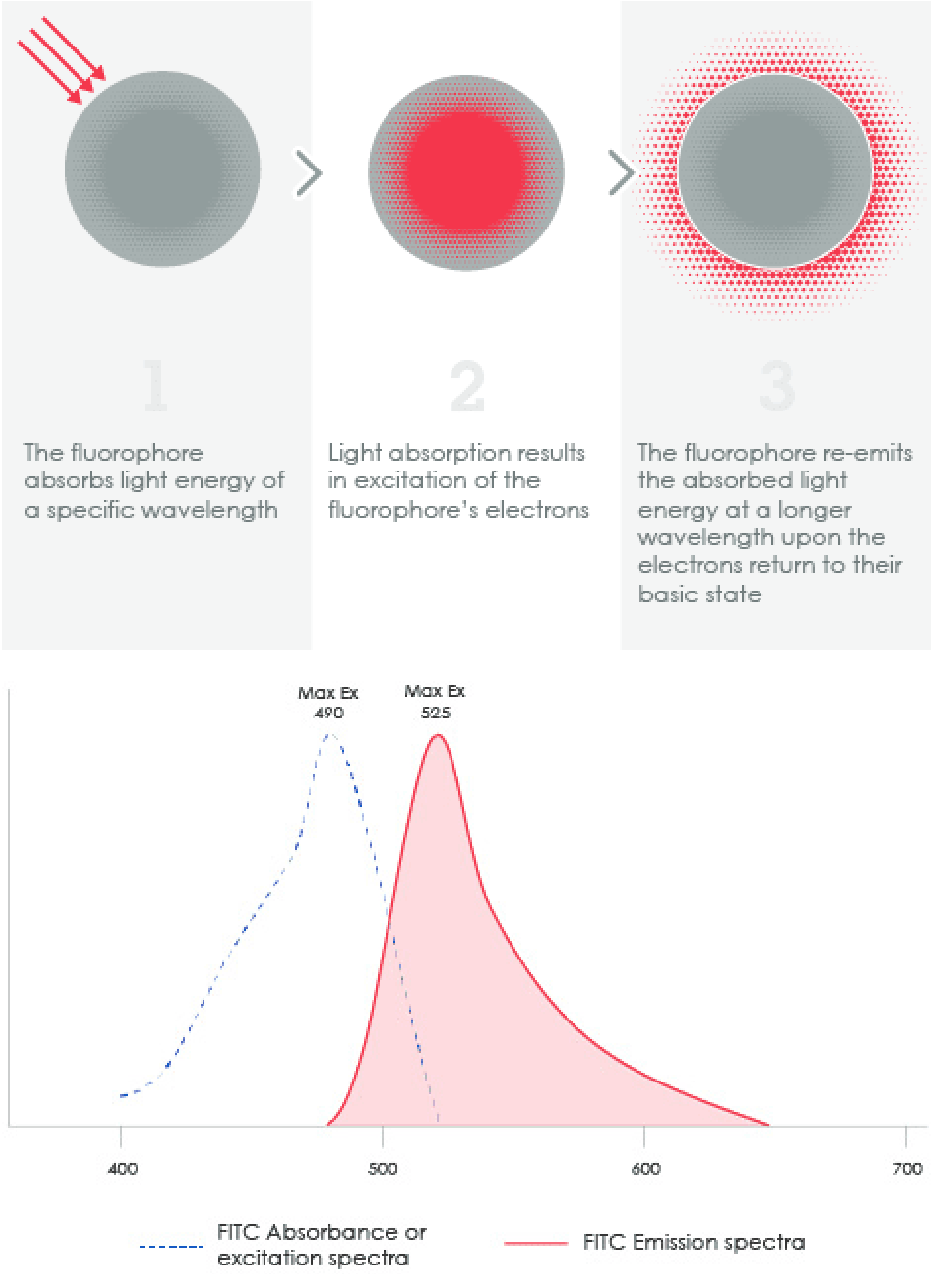


Fig. 2 Fluorophores behaviour in the presence of light at specific wavelengths

B. The fluorophores that absorb blue light and respond with increased energy levels

1. FAD (flavin adenine di-nucleotide)

FAD is considered to be the cellular factor creating the most blue light absorption epithelial fluorescence.

FAD is a coenzyme (a substance that helps enzymes in their catalytic action or acceleration of chemical reactions) active in the Krebs cycle (the cellular metabolic cycle essential for producing energy in aerobic organisms).

When a cell is active in the cellular metabolism there is a lower concentration of FAD since it is consumed faster. Therefore, in the presence of a tumor or dysplastic cells, characterized by greater cellular activity, there is an exponential increase in metabolism which causes a reduction in FAD resulting in a decrease in fluorescence.

WHAT YOU WILL SEE WITH GOCCLES: by observing with the Goccles eyewear and a blue light (a common curing light), the areas with cells involved in a carcinogenic process, we would see a reduction in fluorescence of the tumor area that will appear as a dark area (darker than the healthy green colored oral mucosa).

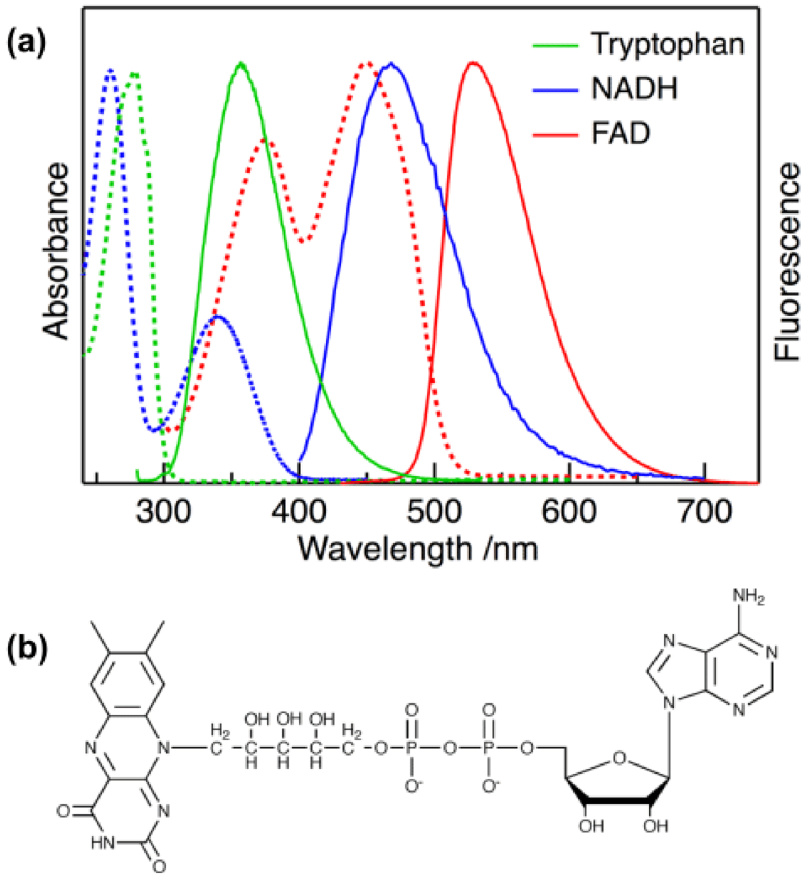


FIG. 3 (a) Absorption (dotted lines) and fluorescence (continuous lines) spectra of self-fluorescent species note the wavelength of the absorption and fluorescence peak of the FAD. (b) Chemical structure of FAD.

2. Collagen

In the stroma, under the epithelial layer, the greatest contribution to fluorescence is made by collagen and it's organization in dense bundles. This allows maintainence of structural integrity, whose structure density involves a fluorescence in blue light.

With the appearance of dysplasia or tumor, the collagen matrix breaks down resulting in a decrease in fluorescence of the stroma.

WHAT YOU WILL SEE WITH GOCCLES: When observing with the Goccles eyewear (and blue light), the areas with a tumor or dysplastic stromal cells, we would see a reduction in fluorescence of the affected area that will appear as a dark area (darker than the mucous membrane) healthy oral color.

3. Keratin

Keratin is a structural protein that exhibits fluorescent character when excited by blue light. Some areas of the oral mucosa are naturally keratinized such as gingival attack and hard palate.

Other tissues in the oral cavity can keratinize (hyperkeratosis) and show an increase in fluorescence as a result of chronic irritation (often of traumatic origin) or part of a pathological process such as leukoplakia. So in the presence of keratinization, we would see a hyper-fluorescence.

WHAT YOU WILL SEE WITH GOCCLES: When observing with the Goccles eyewear (and blue light), an area of hyperkeratosis, we would witness a hyper-fluorescence of the affected area that will appear brighter than the healthy oral mucosa of green colour.

4. Porphyrins

Porphyrins are substances associated with bacteria and give orange-red fluorescence when illuminated by blue light. They are normally observable in the space of the tonsil crypts, on the back of the tongue or in the cracks of the dorsal surface of the tongue. Therefore, in the presence of biofilm, illuminating the mucosa with blue light, we would see an area that will appear orange-red on visual examination by eye.

WHAT YOU WILL SEE WITH GOCCLES: When observing a biofilm with the Goccles eyewear (and blue light), we would see lighter areas of the healthy oral mucosa (green in color). In this case, it is important to check with the naked eye which will show a red-orange fluorescence where porphyrins are present.

5. Fibrin

Fibrin is a fibrous protein involved in blood clotting which can be found in the oral cavity following ulceration. Fibrin normally appears red in white light and the presence of fibrin due to mucosal ulceration is a symptom of an anomaly. Fibrin, when illuminated by blue light, gives a light rather than dark light response, but care must be taken during screening to observe and evaluate the areas surrounding the fibrin that could potentially mask an underlying fluorescence loss, a symptom of other pathologies in progress.

WHAT YOU WILL SEE WITH GOCCLES: When observing with the Goccles eyewear (and blue light) a fibrin area, we should see a bright area of the mucosa. It is important to observe wherther around this lighter fibrin area there is a dark halo. This would be indicative of another pathology (hidden by the fibrin itself) that manifests itself with a loss of fluorescence not visible if covered by the overlying fibrin.

6. Melanin and Hemoglobina

These two molecules both increase the absorption of light in the oral tissue, such that their presence causes a marked decrease in tissue fluorescence. This manifests itself as a distinct dark area in the middle of the fluorescent green of the oral mucosa. In particular, the blood absorbs all the light (except the red one) and absorbs more light of the shorter wavelength (blue and green). In the case of inflamed or damaged tissues with the presence and accumulation of blood, these will appear darker when illuminated by blue light. The areas pigmented by snow, melanomas or "tattoos" caused by the presence of amalgam, appear dark both on visual examination with the naked eye and with the blue light and the Goccles filter.

WHAT YOU WILL SEE WITH GOCCLES: When observing with the Goccles eyewear (and blue light) with inflamed cells (haemoglobin) or pigmented cells (nevi, melanomas), we would see a reduction in fluorescence that will appear darker than in healthy areas that appear green.

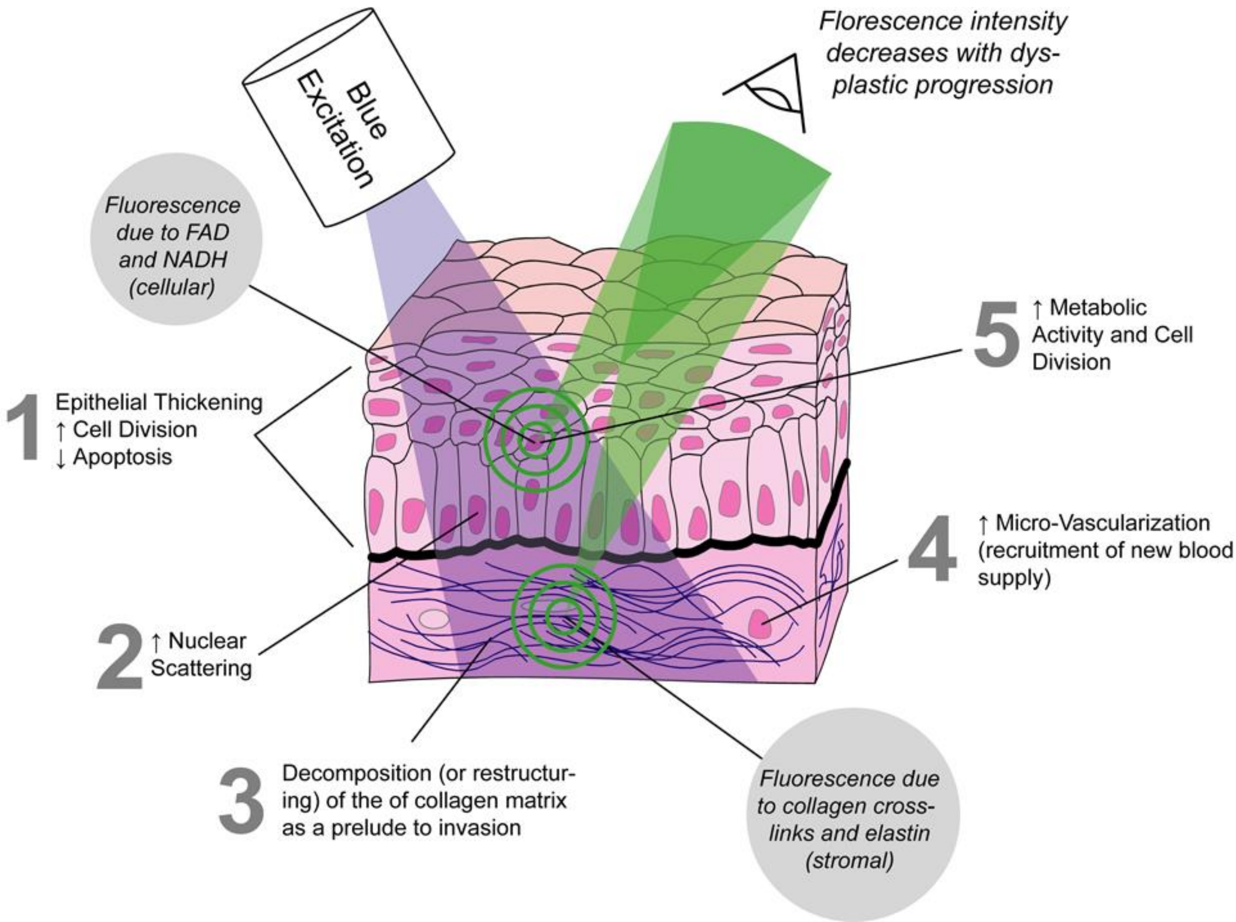


Fig. 4 Tissue fluorescence and dysplastic progression.

The autofluorescence of the tissues (or native fluorescence) is essential for the early diagnosis of oral cancer and the identification of the margins for surgical resection of tumors.

The stages of dysplasia and the use of fluorescence in preventive screening

Precancerous epithelial lesions typically begin below the tissue surface and grow to occupy the entire epithelium.

Different stages of dysplasia are recognized:

- Mild

- Moderate

- Severe

When dysplasia takes the entire epithelium it is called carcinoma in situ and is classified as 'invasive oral squamous cell carcinoma' when it also occupies the basement membrane.

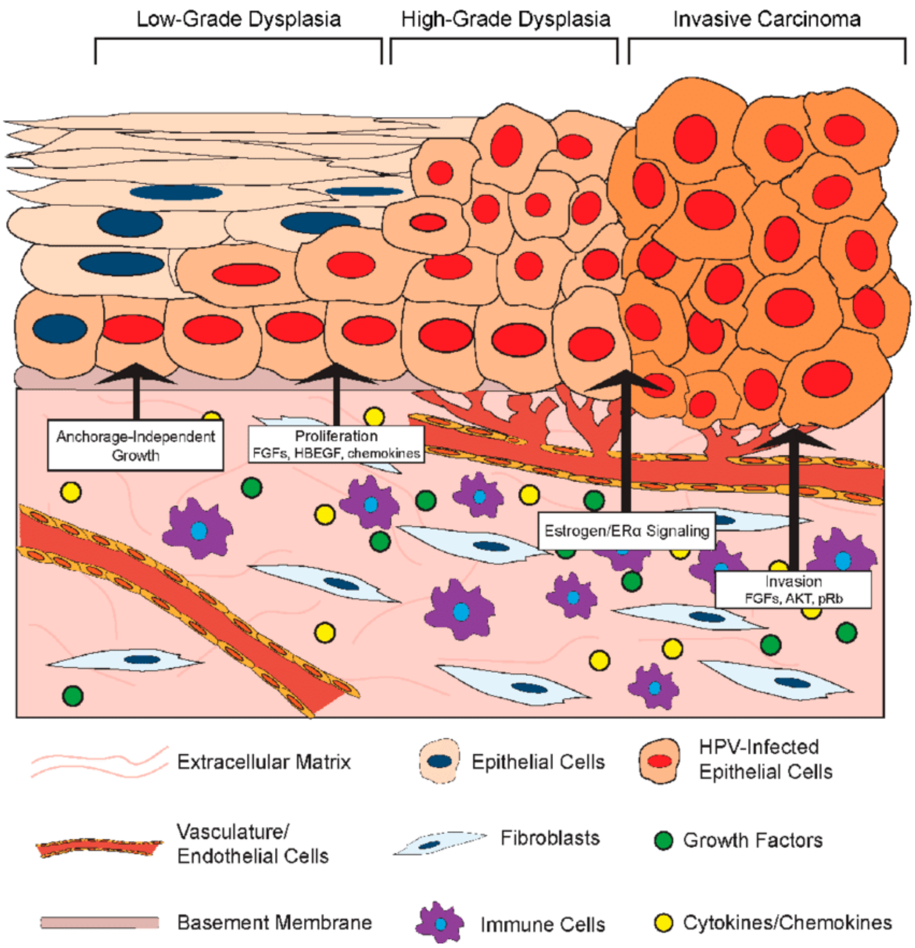


Fig. 5 Degrees of dysplasia in oral cancer

Oral cancer is increasing worldwide in incidence and mortality rates, therefore the implementation of screening for early diagnosis is vital to reduce the morbidity and mortality associated with this pathology.

The ideal time to detect a tumour lesion and to have surgery is in the pre-cancerous stage where the prognosis for the patient is the best.

Let's review, then, how dysplasia and tumors of the oral cavity can result in a decreased intensity of fluorescence in blue light and optical filter screening with the Goccles eyewear.

Essentially the loss of fluorescence in the presence of tumor processes is possible in 4 possible conditions:

1. The increased metabolic activity of dysplastic cells in the epithelium causes a decrease in FAD with a consequent reduction in fluorescence.

2. The breakdown of collagen matrices as a prelude to the advancement of cancer cells leads to a decrease in fluorescence.

3. Morphological changes that can occur in dysplastic cells involve an increased scattering or scattering of light in the epithelial layer. This increases the backscattering of blue light which excites the fluorescent component of the cells, with a consequent evident decrease in fluorescence (visible phenomenon even with the naked eye).

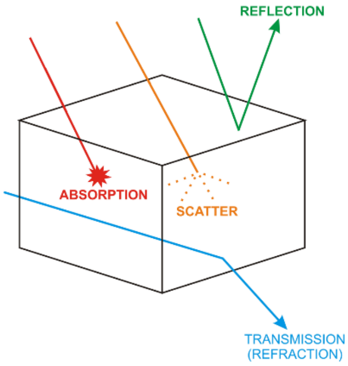


Fig. 6 The behaviour of light in the presence of an object

4. An increased intake of blood due to an increase of activity of dysplastic epithelium cells will result in a greater microvasculature of the stroma and therefore a greater absorption of light by the blood. Abnormal growth of dysplastic cells in the epithelium also leads to an increase in blood flow to the affected area as a normal physiological response. These two processes will result in a diminished fluorescence (phenomenon visible even with the naked eye).

The loss of fluorescence, however, is not only due to dysplasia or oral cancer, and this represents a limitation of this technique. Various causes can be associated with different types of lesions, as well as areas of prominent vascularization, areas of inflammation and excessive melanin pigmentation, all attributable to a loss of fluorescence. For this reason, in the presence of areas with loss of fluorescence, it is essential to follow the course over the next 2 to 4 weeks to obseve the regression or persistence of the observed phenomenon.

Furthermore, it should not be overlooked that tissue fluorescence screening for oral cancer is part of a clinical examination that includes the patient's medical history with the risk assessment of the tumor, head-neck palpation and a first visual screening with the naked eye illuminated by white light.

The only way to accurately determine a diagnostic finding is with a biopsy and histopathological examination that allows identification of the possiblity of a false positive.

When performing tissue fluorescence screening, it may be helpful to have clinical imaging support from a pathologist or surgeon who has experience with this method, GOCCLES offers this support. For information, please send an email to info@goccles.com or visit www.goccles.com

References

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