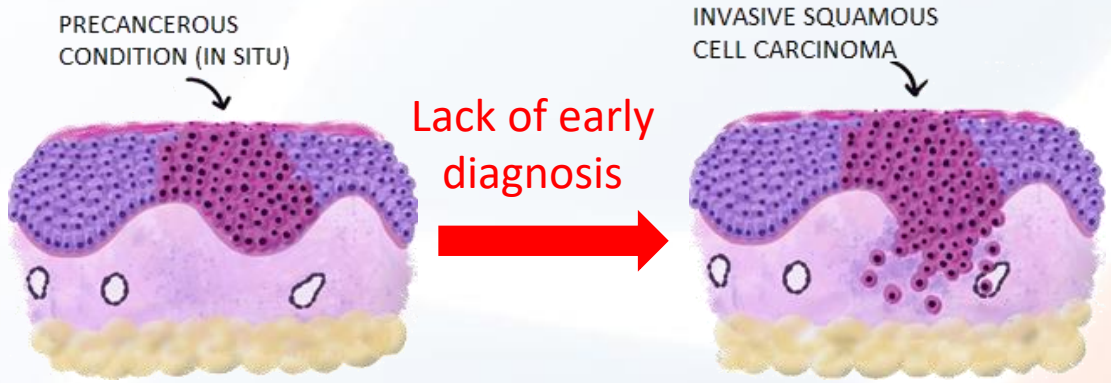
FLUORESCENCE IMAGING METHOD FOR SCREENING PATHOLOGICAL CONDITIONS OF THE ORAL MUCOSA

Ekaterina Bryanskaya, Vitalina Zakrzhevskaya, Andrey Dunaev
Research and Development Center of Biomedical Photonics, Orel State University, Russia

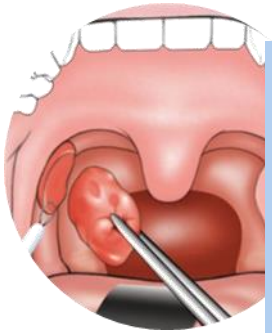
SARATOV FALL MEETING XXIX
September 29 - October 3, 2025, Saratov

- MALIGNANT NEOPLASMS OF THE ORAL CAVITY OCCUPY **THE 6TH PLACE** AMONG THE MOST COMMON TYPES OF CANCER

- ANNUALLY >**600 THOUSAND** NEW CASES



APPROACHES TO THE DIAGNOSIS



Biopsy

Disadvantages:
- Morbidity and invasiveness
- Time costs and cost of the procedure
- The importance of determining the exact lesion area

Fluorescent diagnostics

Disadvantages:
- Visual assessment of fluorescence intensity

!

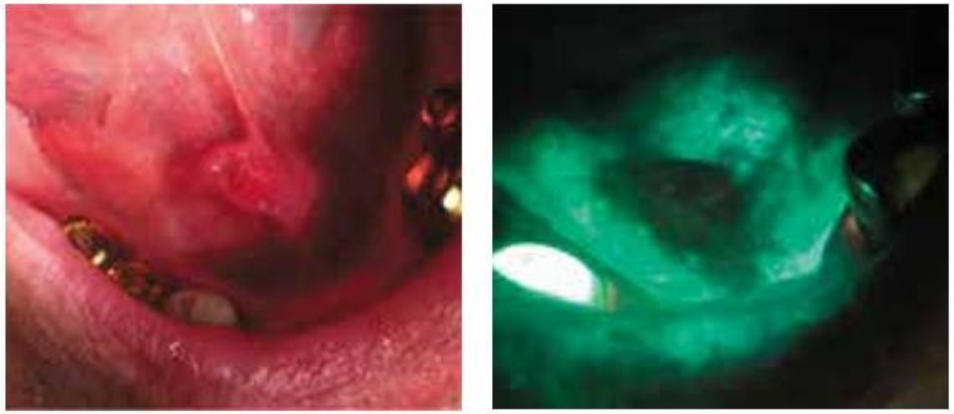
SUBJECTIVITY OF RESULTS INTERPRETATION,

THE NEED FOR A HIGHLY QUALIFIED DOCTOR

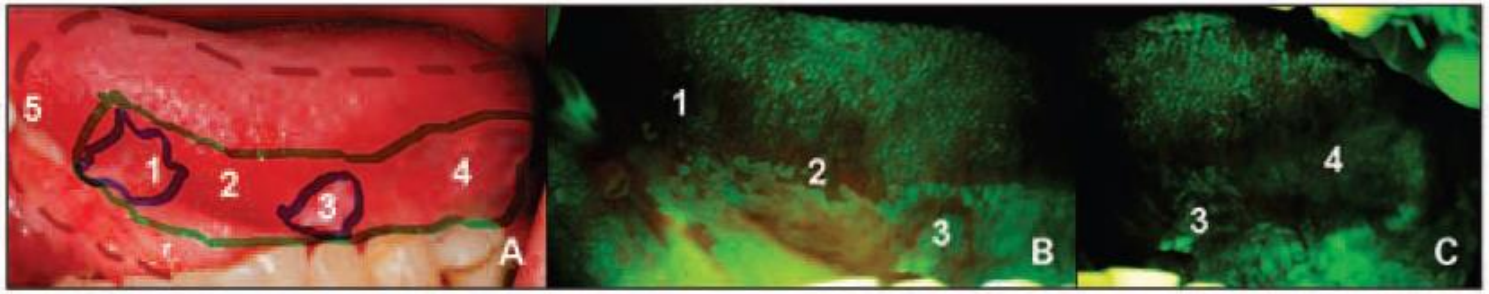
The purpose of the study is to develop a technology of fluorescence imaging for diagnosis pathological changes of oral mucosa conditions, which would be safe and applicable in the framework of public health screening.

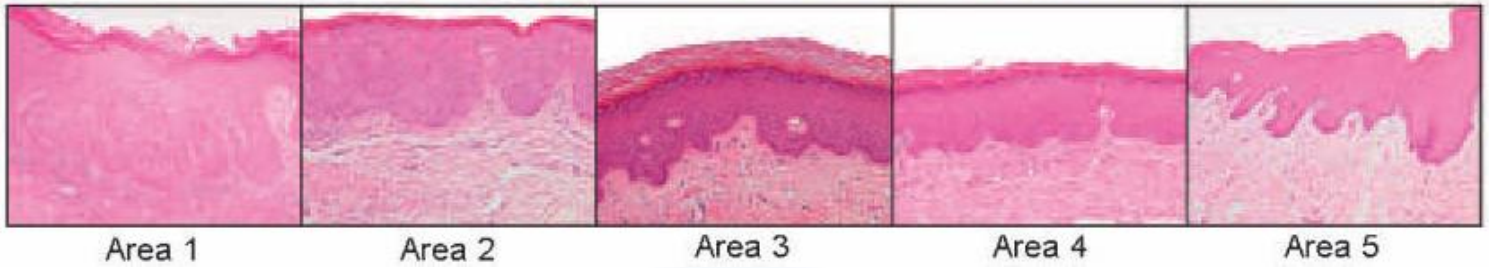
Oncological tissues of the oral cavity have **the lowest level of autofluorescence** compared to healthy tissues [2-4]

[1] Bulgakova N. N. et al. Russian Dental Journal, 2015
[2] Cioban C. V. et al. Romanian Journal of Stomatology, 2022
[3] Lima I. F. P. et al. Photodiagnosis and Photodynamic Therapy, 2021
[4] Koch F. P. et al. Clinical oral investigations, 2011



Oral cavity cancer





[5] Poh CF. et al. Cancer Prev Res (Phila), 2009

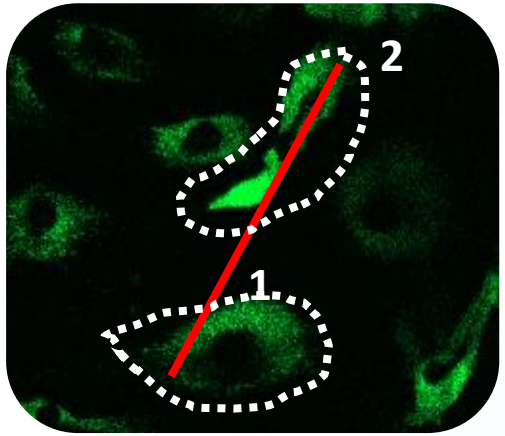
1 and 3 are clinically obvious and demonstrate loss of autofluorescence,
2 and 4 are not clinically obvious, but have a loss of autofluorescence,
5 is located outside the clinical tumor and the boundaries of autofluorescence loss.

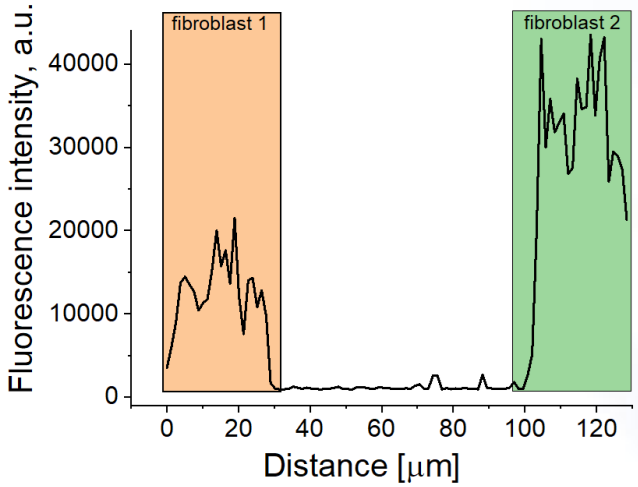
Histology:
1 - carcinoma in situ;
2 - severe dysplasia;
3 - moderate epithelial dysplasia;
4 - epithelial dysplasia of mild and moderate severity;
5 – absent of dysplasia

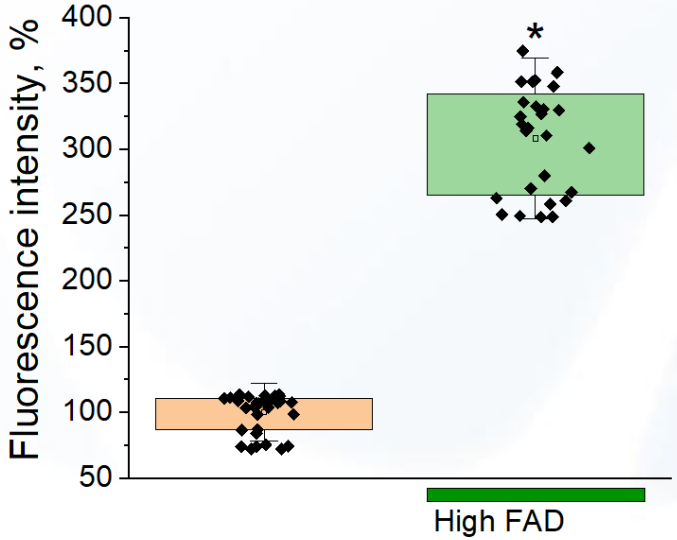
Can a high level of FAD autofluorescence indicate cell pathology and, possibly cell death?

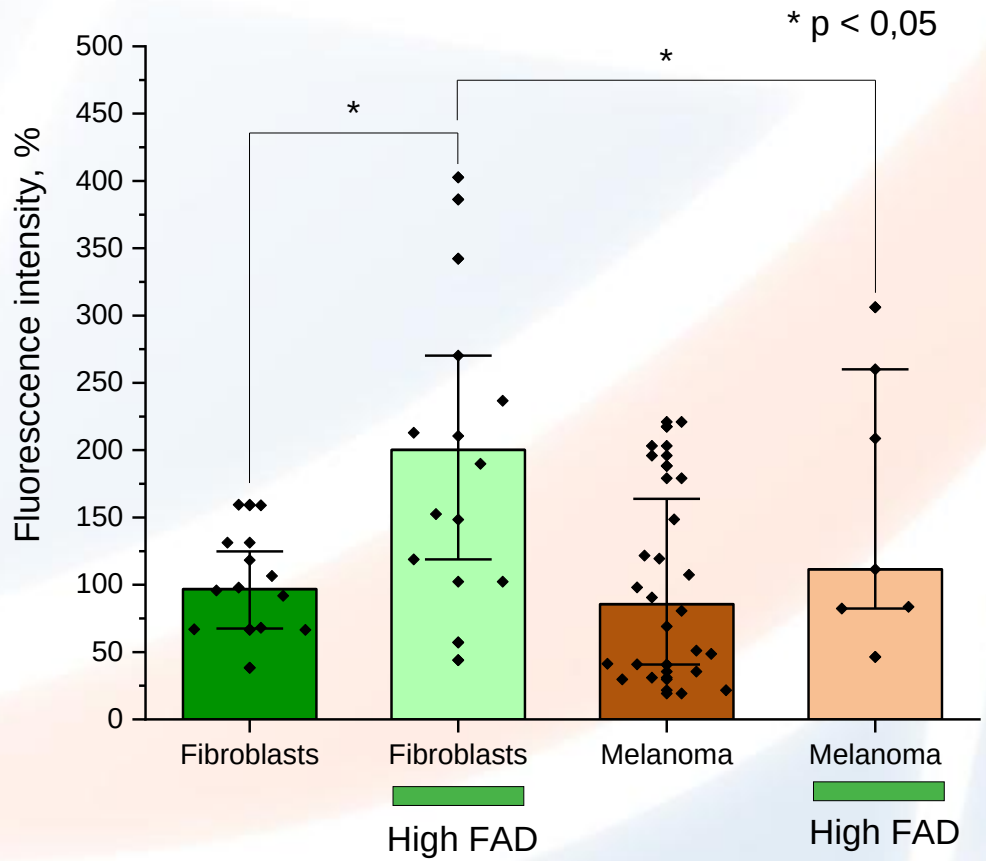
ZEISS LSM 900 confocal microscope
with the Airyscan 2 system (Carl Zeiss AG, Germany)
Excitation wavelength: 488 nm,
Fluorescence detection interval: 505-550 nm

- The cell line of human fibroblast culture;
  - B16 and B16/F10 melanoma cells
(*cultivation before the study is 20 days*)

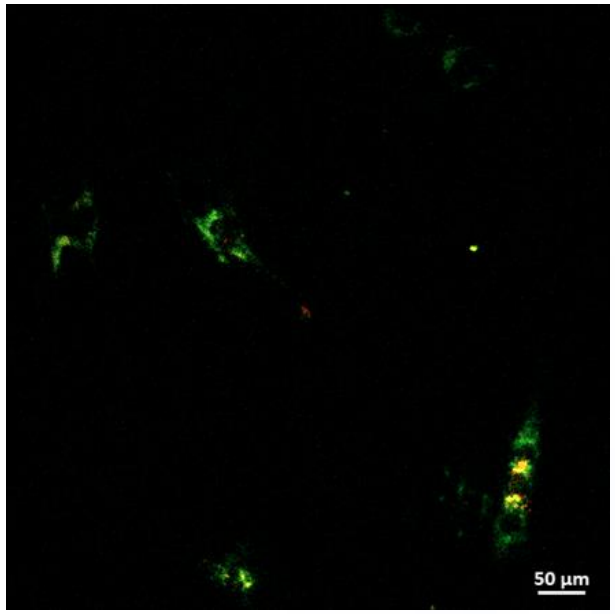


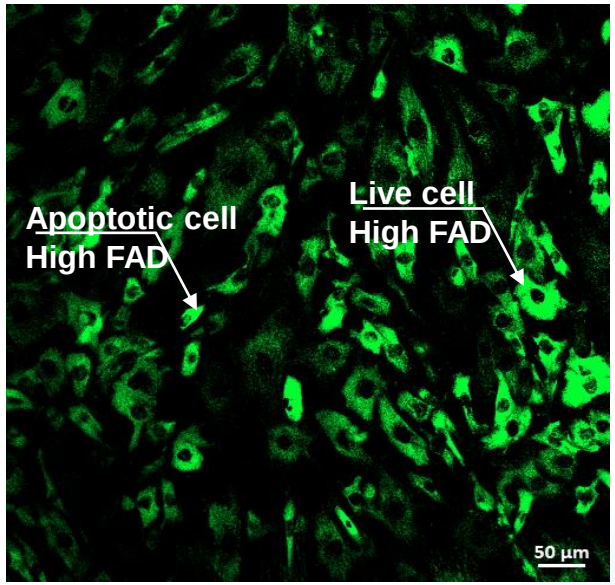


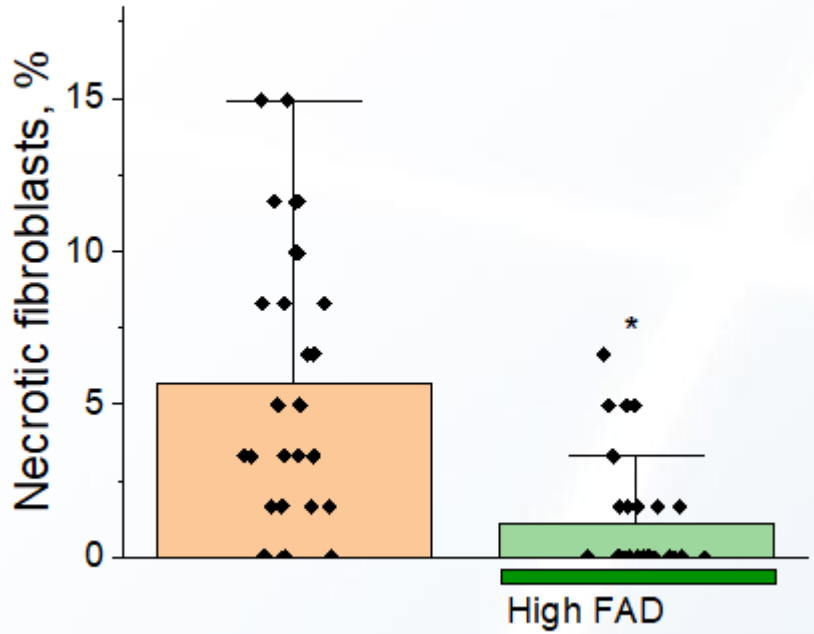




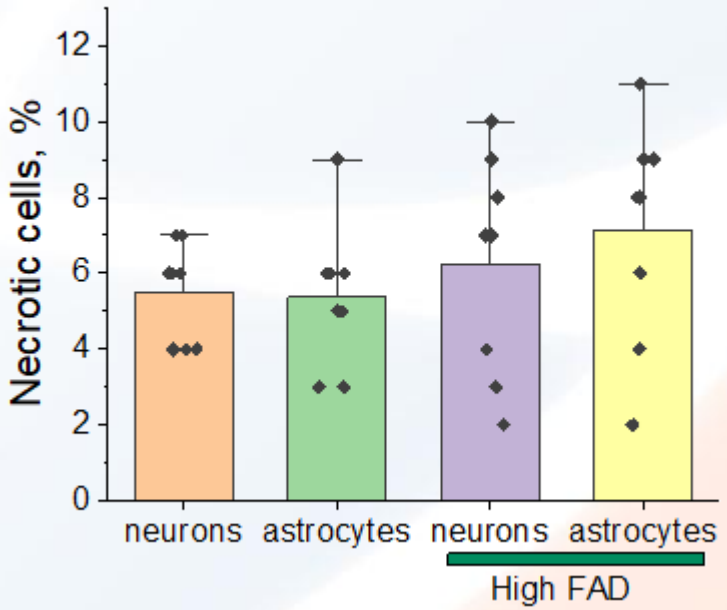
High levels of FAD are not associated with necrosis or apoptosis at the time of measurement

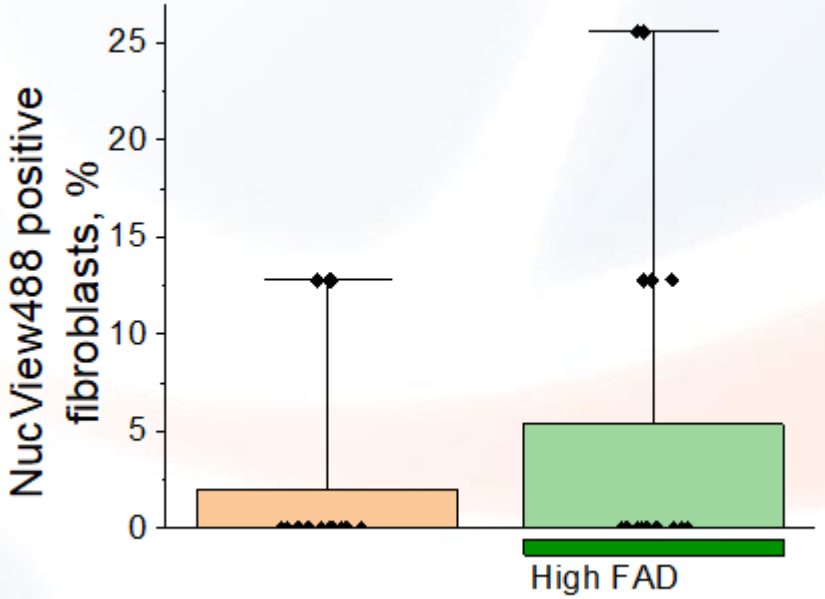




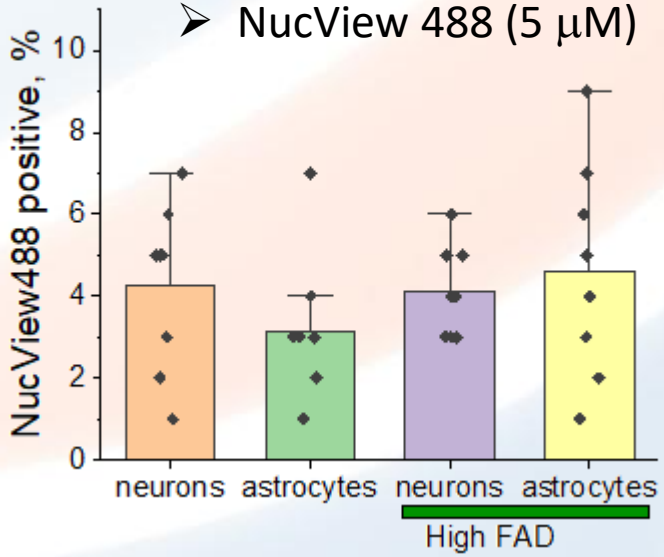


➢ PI (10 µM)

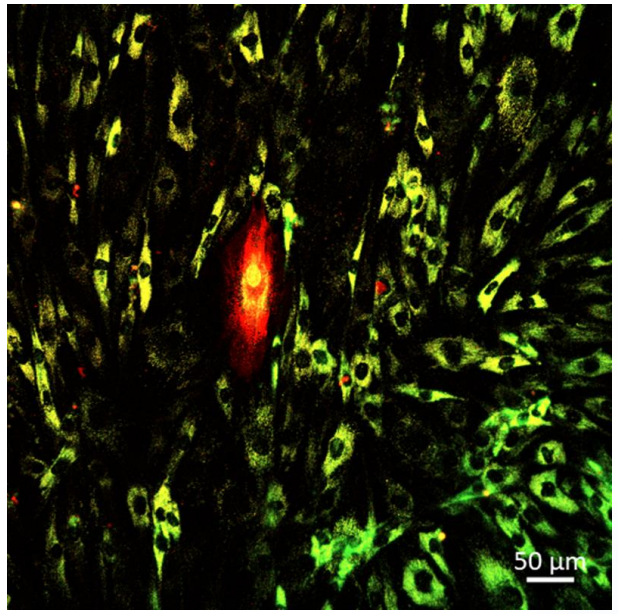


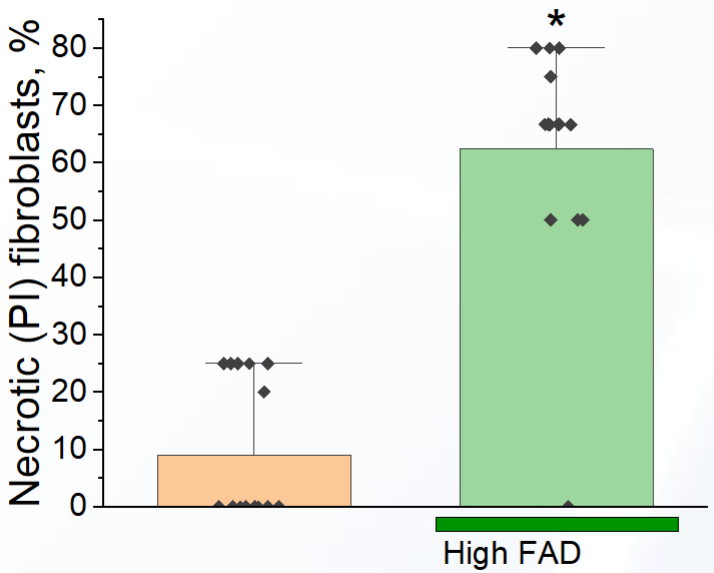


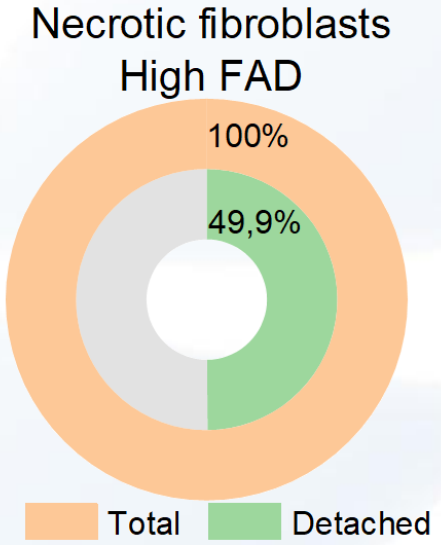
➢ NucView 488 (5 µM)

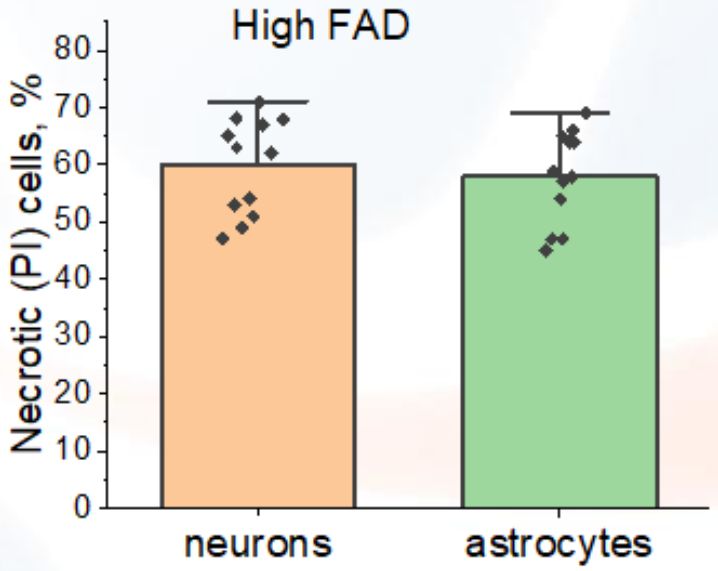


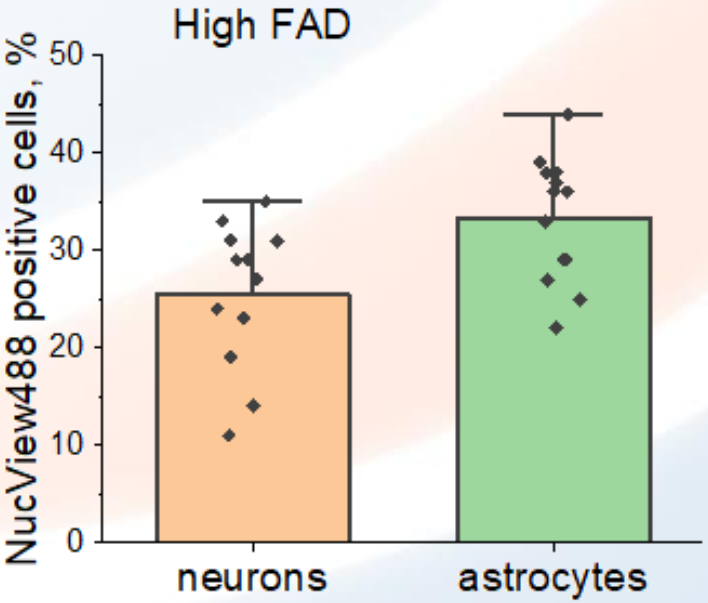
High levels of FAD lead to cell death in the following 24 h



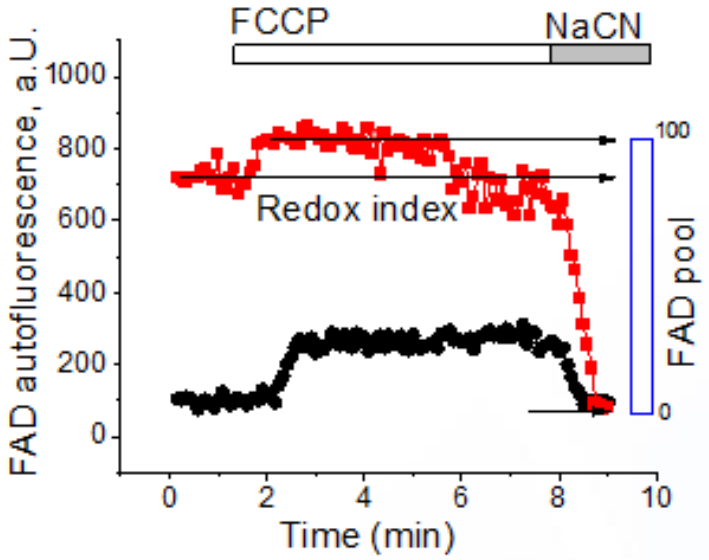


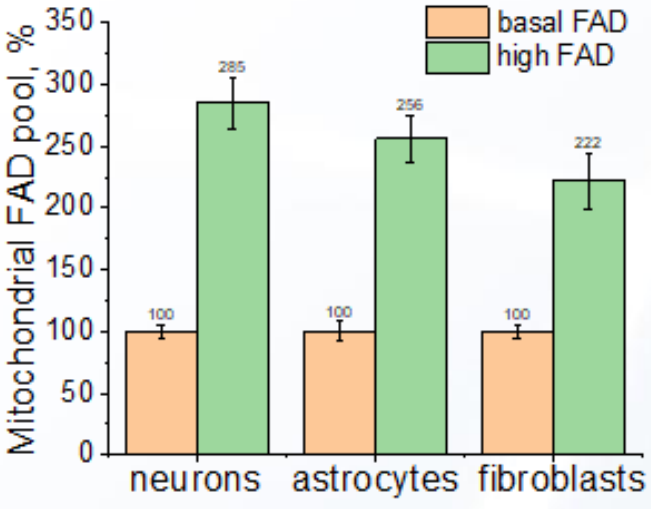


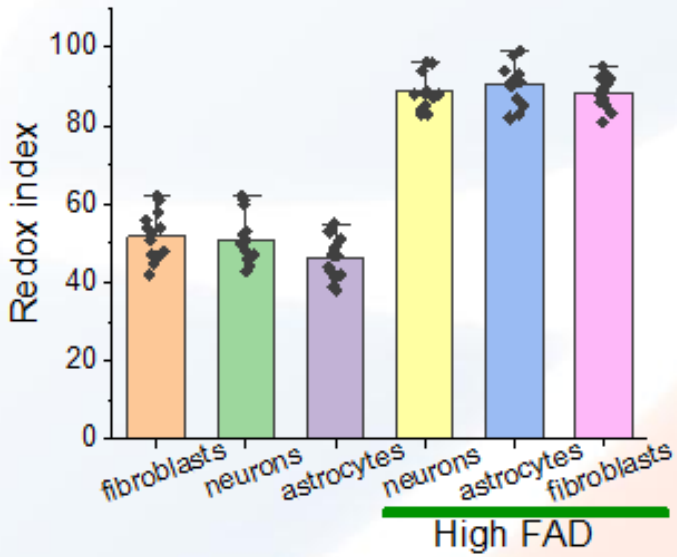


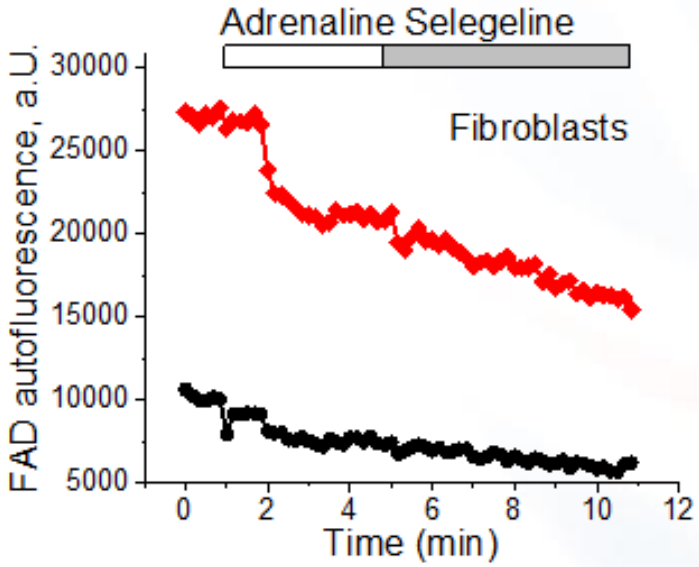


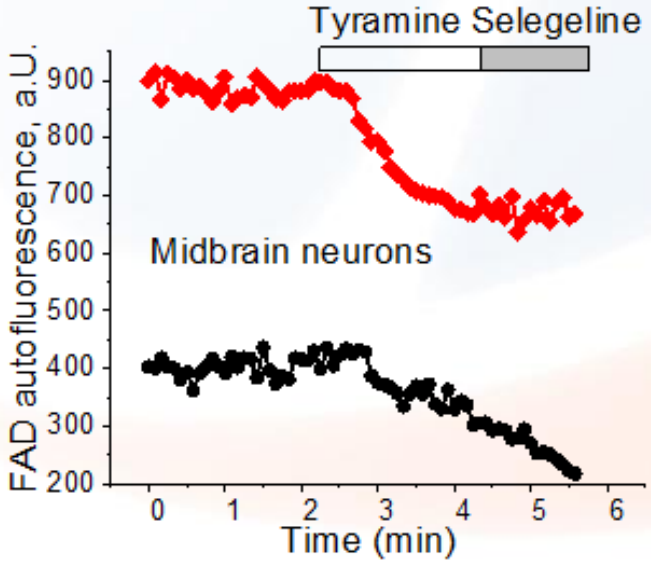
High FAD signal is due to increased levels of FAD turnover in mitochondrial complex II and increased MAO activity

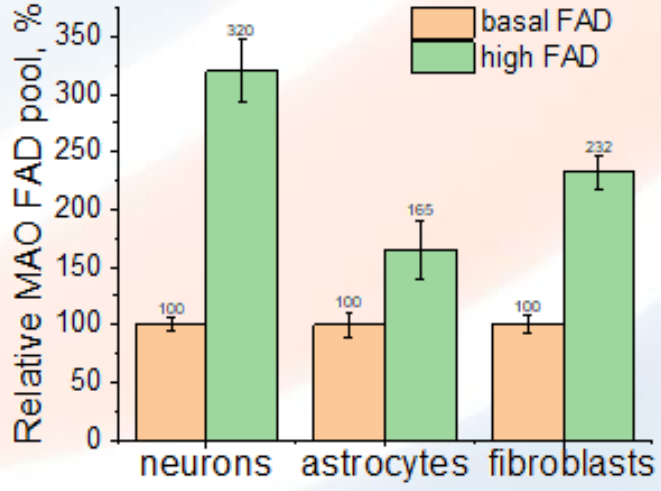








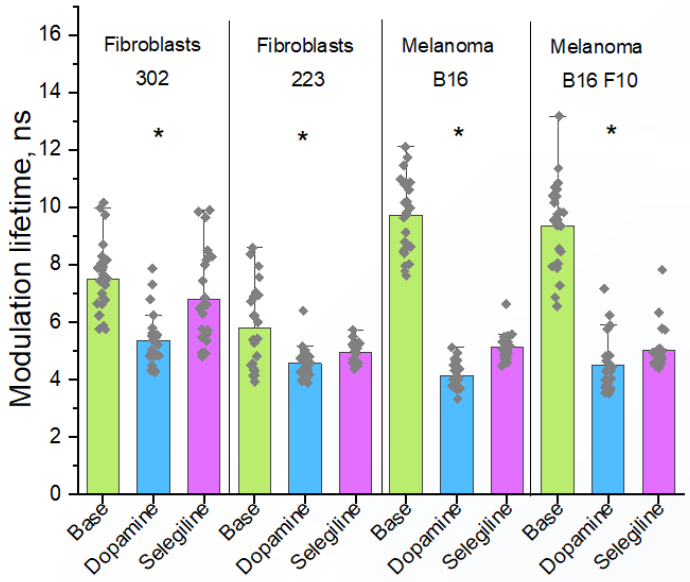


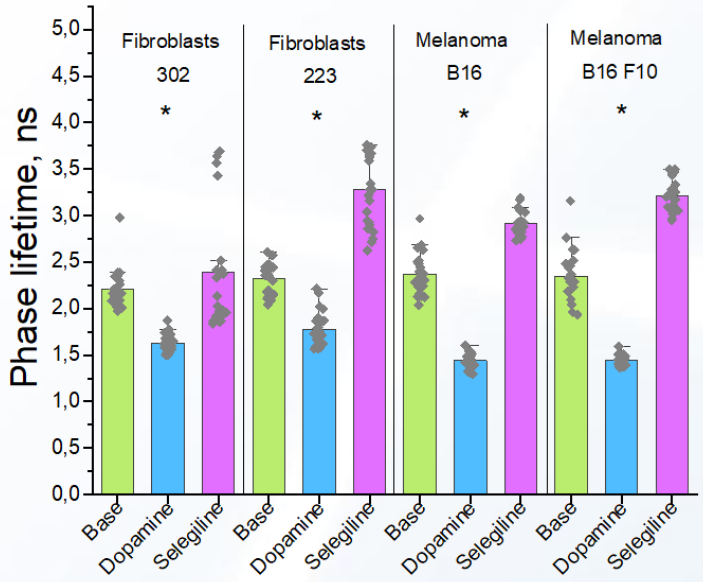


Measurement of fluorescence lifetime using FLIM

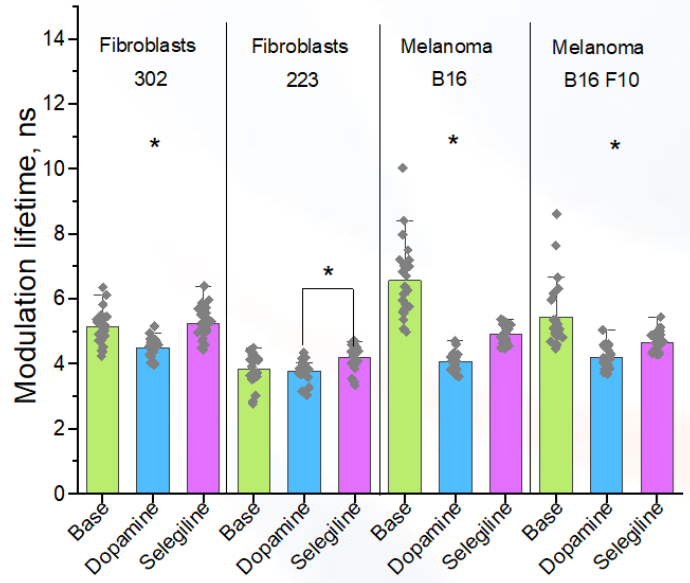
*p <0.05

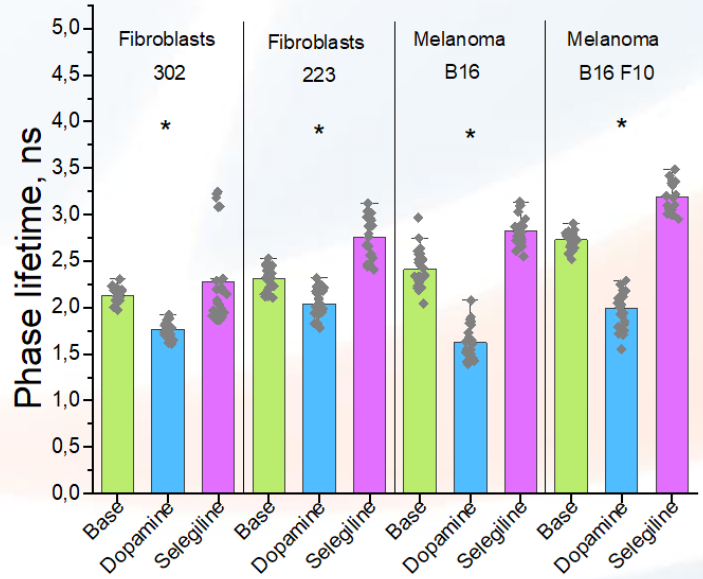
High intensity cells





Low intensity cells



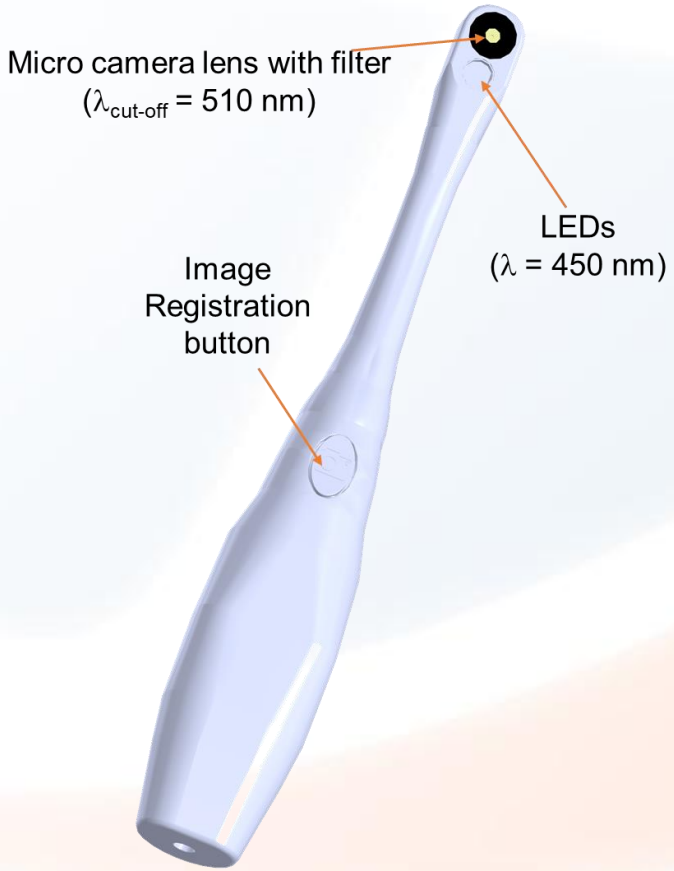


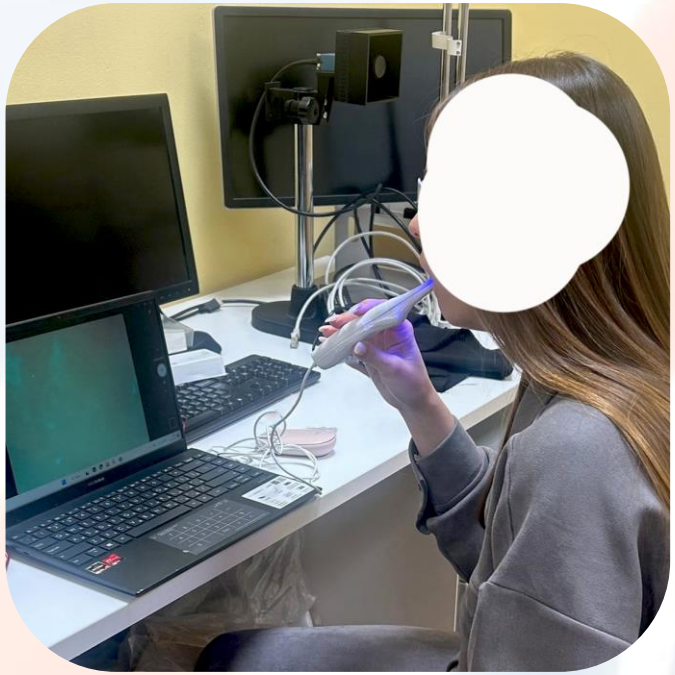
The high intensity of FAD autofluorescence in fibroblast cells is associated with the **contribution of short-lived protein molecules**, which can serve as an early marker of apoptosis.

The fluorescent imaging channel for oral mucosa diagnosis

Specialized medical and technical requirements:

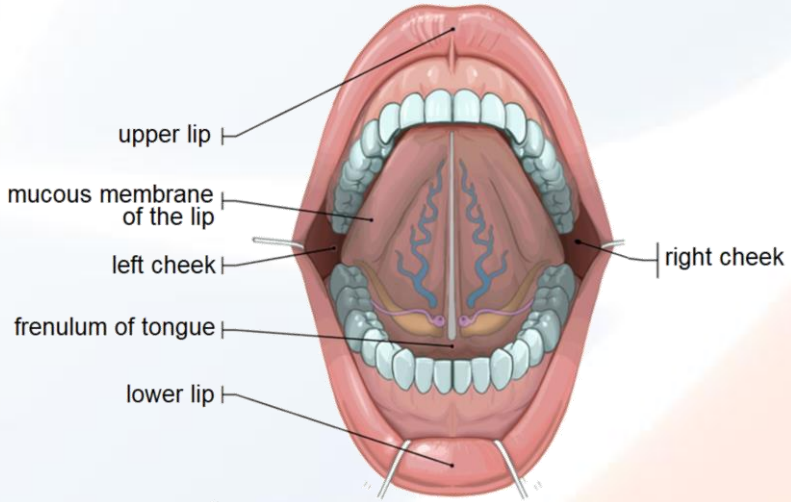
- excitation wavelength – 450 nm,
- wavelength of the cut-off filter– 510 nm

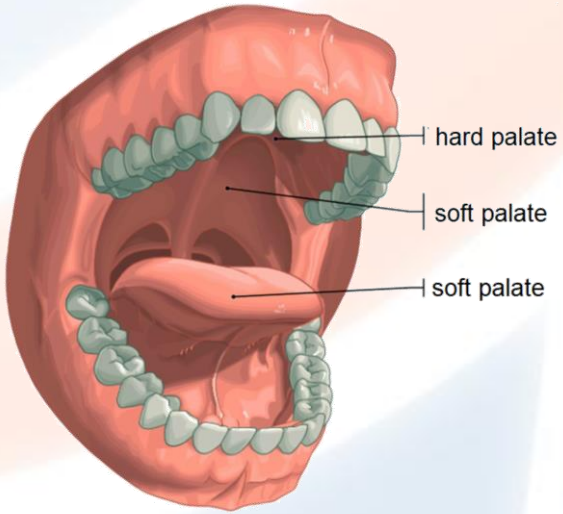




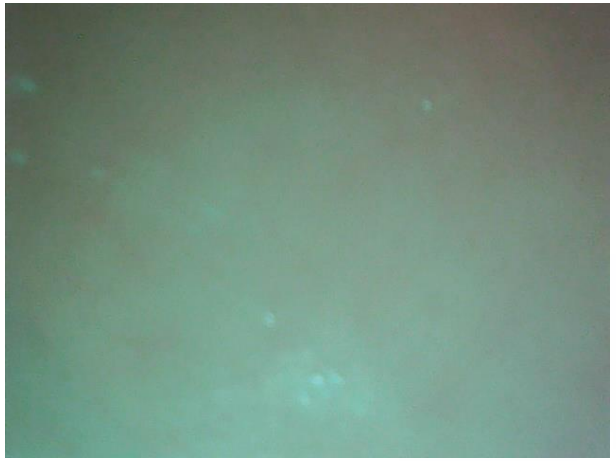
The fluorescent imaging channel for oral mucosa diagnosis

I Study preparation 1. Connecting the device to PC. 2. Patient registration in the database. 3. Filling out and signing the informed consent and the health questionnaire. 4. The patient's location in a sitting position. 5. Disinfection of the device.	**~ 2 min**
II Conducting study. Image registration in the following areas: ▪ mucous membrane of the left cheek, ▪ mucous membrane of the right cheek, ▪ dorsal surface of the tongue, ▪ hard palate ▪ soft palate, ▪ mucous lips, ▪ red lip border	**~ 5 min**
III Image analysis in ImageJ program 1. Mark on the diagram: + area with low autofluorescence, ** area with ultra-bright autofluorescence. 2. Mark 5 ROIs in each research area; 3. Calculate the average autofluorescence intensity value separately for each study area; 4. Displaying the result in the protocol.	**~ 7 min**

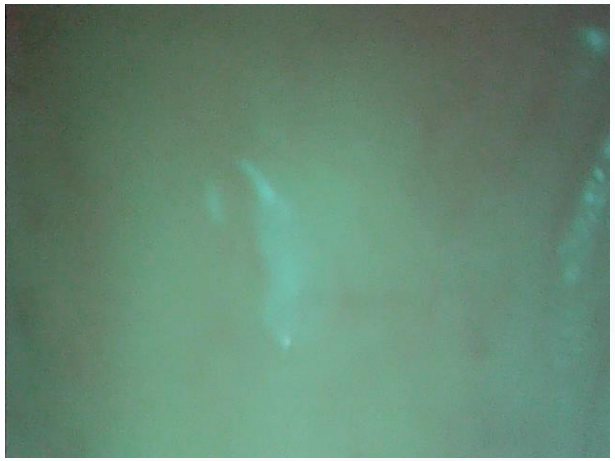




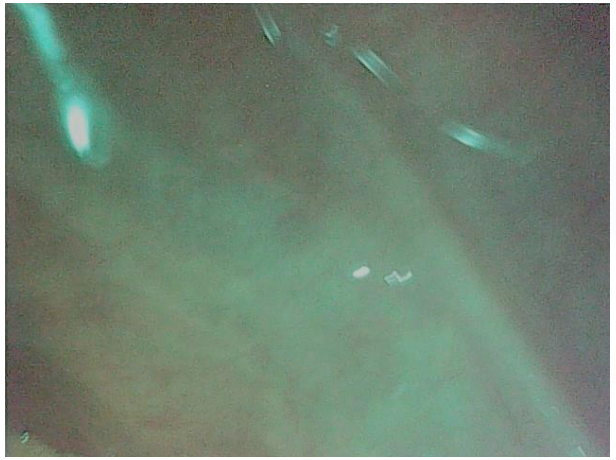
Example of autofluorescence images registration of a conditionally healthy volunteer



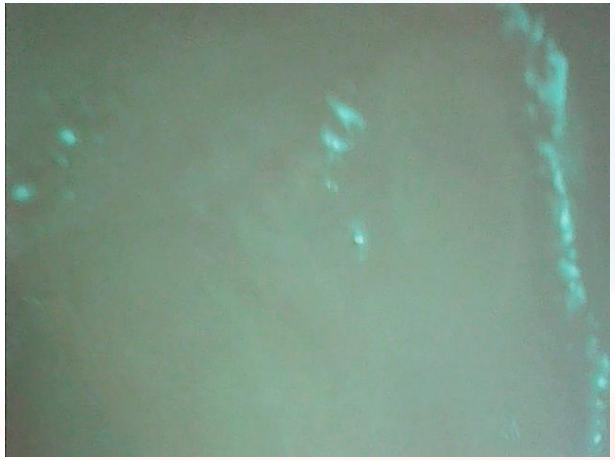
mucous membrane of the right cheek



hard palate



lower surface of the tongue



mucous membrane of the left cheek

- **The normal mucous membrane** has a green glow, while there were no areas with ultra-bright or ultra-low autofluorescence.
- The mucous membrane of the sublingual area had a **green-red autofluorescence**, which indicates the **presence of plaque**, which includes microorganisms and their waste products.

CONCLUSIONS

- The obtained results showed the prospects of using the fluorescence imaging method for the diagnosis of oral mucosa pathologies. It has been shown that the **high level of FAD can serve as a marker of a inflammatory process**, and an **the lowest level is a marker of cancer**;

- The device developed can be used in public health screening, during the initial examination of patients by a dentist, an ENT doctor of head and neck oncology, as well as in evaluating the effectiveness of treatment;

- The use of convolutional neural networks will allow the binary classification of oral mucosa condition into classes of presence or absence of pathology.

RSF Russian Science Foundation

This study was funded by
Russian Science Foundation № 24-75-00144.

Contact details:
Ekaterina Bryanskaya
Researcher
R&D Center of Biomedical photonics
CEO «DiaphanoScan» LTD
Email: **bryanskayae@mail.ru**
www.bmecenter.en

Thanks for the attention!