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Electron Microscopy of Squamous Cell Carcinoma of the Head and Neck

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Squamous cell carcinoma of the head and neck carries a bad prognosis. The most important thing to attain in order to achieve cure is local tumor control. The main therapy available is external radiotherapy, which can be supplemented when necessary with interstitial radiotherapy, chemotherapy and surgery. In this paper we have evaluated specimens from thirty-five patients with squamous cell carcinoma of the head and neck. The specimens were taken before therapy in the process of staging. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analyses were made. On SEM, the parameters analyzed were the amount and appearance of microvilli, filaments and blood vessels. On TEM scoring was made of the filaments, desmosomes, nuclei, nucleoli, mitochondria and blood vessels.

Scoring of the samples showed a difference between the group with recurrent disease (N=10, group A) and the group with local tumor control (N=25, group B) regarding blood vessels and intracellular filaments.

Multi-photon Excited Fluorescence Spectra of Common Bio-probes

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Fluorescent probes are commonly used in biological fluorescence microscopy for tracking specific structures and subcellular compartments, and for indicating cellular ionic conditions. Recent development in multi-photon fluorescence microscopy has greatly expanded the usage of fluorescent probes in biomedical research. Considering its non-linear nature, two-photon excitation may generate very different fluorescence spectral response in the sample when compared with single photon excitation[1,3,4]. It is thus necessary to measure the two-photon spectra of various fluorescent probes, so that two-photon fluorescence microscopy may be performed effectively and the images properly interpreted. This report represents the second installment of a continued effort in characterizing the multi-photon fluorescence spectra of commonly used bio-probes[2].

Two-photon fluorescence spectra excited with near infrared at 780nm were obtained with a SpectraPro-500 spectrophotometer (Acton Research) equipped with a TE-cooled PMT and coupled to a Spectra-Physics Tsunami Ti-sapphire laser pumped by a Coherent Verdi solid-state laser operated at 85MHz, 100fs pulse. The 1240nm infrared (IR) excitation was obtained from a Spectra-Physics Millianin IR (1064nm) pumped Chromium-doped Forsterite laser (built by CKS) operated at 120MHz, 130fs pulse. A cooled CCD array spectrophotometer (Acton Research) was used for spectral detection. An Olympus BX microscope trinocular head and an epi-fluorescence beam-splitter housing were modified for the measurements. A 740nm dichroic beam splitter was used for separating the excitation beam and the fluorescence emission. In addition, two IR cut-off filters (Edmond Scientific, Cat. K53-710) were installed in front of the entrance slit of the monochromator to reject scattered IR from the sample. A 4x microscope objective was used to focus the pump beam into a 0.3ml microfuge tube. Two-photon fluorescence images were taken with an Olympus water immersion objective (UPLANApo 60x W-PSF, NA=1.2) on an Olympus Fluoview inverted confocal microscope. The same Ti-sapphire laser described above was used for microscopy. A 740nm short-pass dichroic beam splitter was installed in the excitation path of the confocal scanning unit.

Figures 1(a-f) show two-photon pumped fluorescence spectra of six commonly used bio-probes. Measurements of DAPI (Fig. 1a), Hoechst 33258 (Fig. 1b) and Syto 17 [Fig. 1c (Ex=780nm) and Fig. 1d (Ex=1240nm)] were performed with 2μM dye in the presence of 160μg/ml fragmented salmon sperm DNA in TE buffer (10mM Tris, 1mM EDTA, pH7.4). This concentration approximates 50 base pairs of DNA per dye molecule. Methanol solutions of MitoTracker® (Molecular Probe M-7512; Fig. 1e) and LysoTracker Red®(Molecular Probe L-7528; Fig. 1f) were used in the measurement. Spectra e and f were excited with IR at 1240nm, therefore; the MitoTracker® emission is the result of three-photon excitation, while the spectrum of

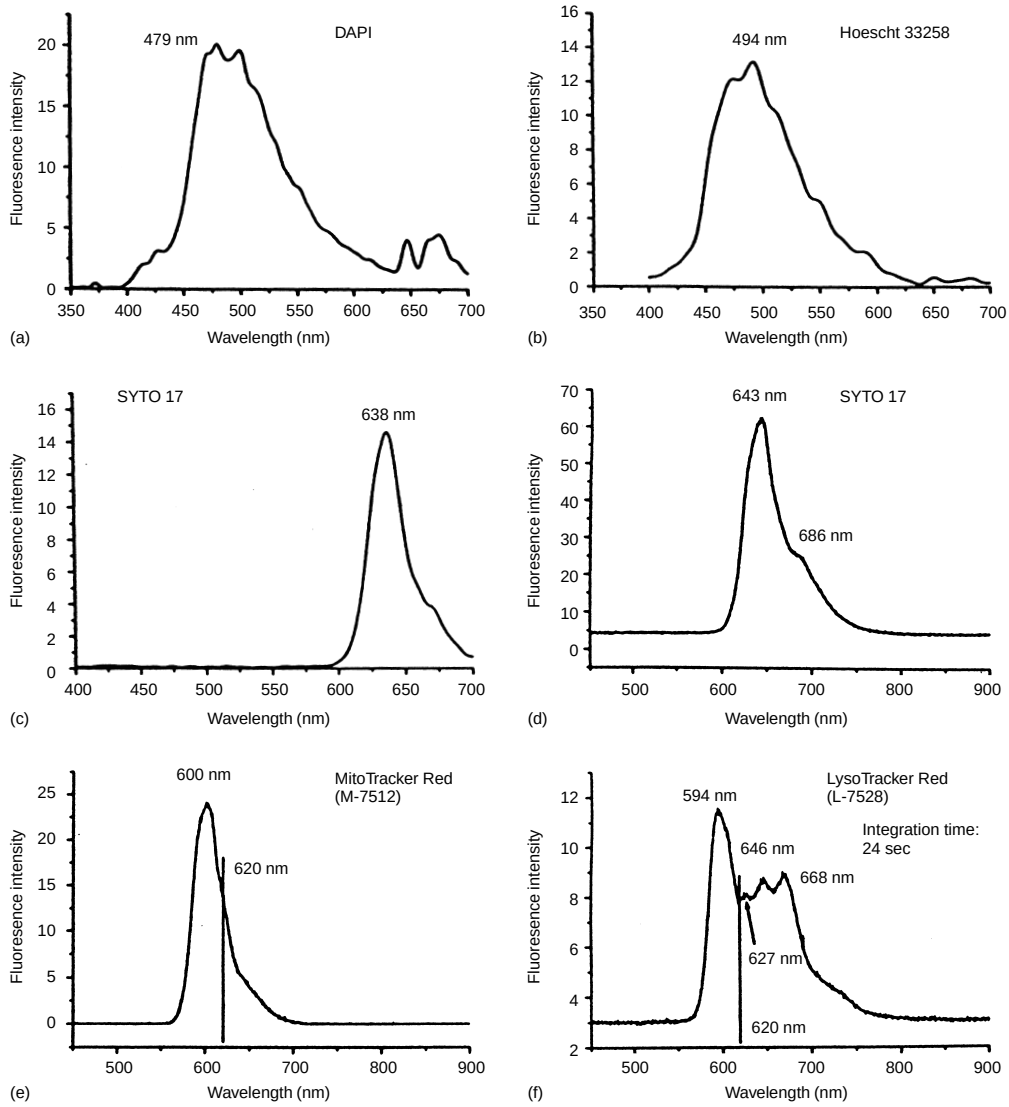

FIG. 1 Two-photon pumped fluorescence spectra of DAPI (a) (Ex=780nm), Hoechst 33258 (b) (Ex=780nm), SYTO 17 (c) (Ex=780nm) and (d) (Ex=1240nm), Mitotracker® (e) (Ex=1240nm), and Lysotracker® (f) (Ex=1240nm).

LysoTracker® is a mixed result of two and three-photon excitation.

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