The confocal laser scanning microscope (CLSM) creates three-dimensional images by reconstructing serial optical sections. Hence, tissues can be investigated as well as single cells within the tissue or cell components. To get an impression of the structural arrangement of viable cells nuclear staining is very helpful. For common fluorescence microscopy nuclei are usually stained with DAPI (4’6-diamidino-2-phenylindol). DNA bound to DAPI is excited with ultraviolet illumination (UV, 359 nm) and emits blue light (440 nm). However, due to apperative reasons most of the confocal laser scanning microscopes are not equipped with an UV laser. The illumination wavelengths are between 488 nm and 650 nm. Our microscope is equipped with three illumination wavelengths (488 nm, 568 nm and 648 nm). A variety of commercial available nucleic acid binding dyes with the specific excitation wavelengths have been developed. Unfortunately the properties of these dyes are rarely comparable with the DAPI staining because they also often stain cytoplasmic RNA [1].

In order to find a nucleic acid stain restricted to the nucleus we tested several nucleic acid dyes on cryosections of human skin (Fig. 1, left): LOLO™-1 iodide (orange), TOTO®-3 iodide (red), LO-PRO™-1 iodide (orange), SYTO® 84 (orange), SYTO® 85 (orange), SYTOX® Green and SYTOX® Orange all from Molecular Probes. Additionally, individual parameters such as dye concentration, incubation time and pre-treatment of the skin were compared.

The dyes showed differences in their staining quality. However, the best results were obtained with the SYTOX® dyes though the orange stain is not as photostable as the green one. SYTOX® stains are impermeable dead cell markers. Therefore after fixation and permeabilization of the cells it can also be used for three-dimensional visualization of cell cultures. In summary, SYTOX® dyes are very suitable nuclei stains for the three-dimensional visualization of tissue sections (Fig.1, right) and cell cultures.

![Figure 1: Simultaneous staining of nuclei with SYTOX® green and actin with Alexa Fluor® 568 phalloidin. Left: Cryosection of human skin. 3-dimensional reconstruction of cryosection of a human sweat gland. Bars: 50 µm](image)