Studies on the Technical Problems of DNA-RNA Cytofluorometry with AO Staining
Masahiro KAMACHI, Katsuki KUSUIKIKI, Takashi FUJIMOTO, Tsukasa ASHIKARA, Osamu TAKEDAKI & Setsuya FUJITA
Department of Pathology, Kyoto Prefectural University of Medicine, Kyoto, and Shiga University of Medical Science, Shiga

To improve DNA-RNA cytofluorometry using AO staining, which is very useful for kinetic analysis of the mixed cell population with polyploidization, we studied the technical problems to achieve standardization of this method, and then tried to apply this method to cell proliferation analysis of various cancers.

The liver cells from the Wistar rats (male, 6 months of age) were examined by DNA-RNA cytofluorometry (NIKON SPM-RF1) for the problems of the smear preparation, quantitative and specific stabilization of AO, and its quantitative cytofluorometry on the stained cells. Our standardized procedures involved: a) to flatten the cells by cytoconcentration with AUTOSMEAR, and b) to modify the AO staining of Barzynkiewicz's 2-step method into 4mg/L in concentration, pH 6.0, 4°C, and 24-72 hours of staining time for liver cells. However, since both the green and red fluorences gradually decreased during staining, we have overcome this adverse phenomenon by to mounting AO-stained smears in the AO solution. Further, the specificity of this fluorescence staining was found to be satisfactory, 96.1% of green and 89.9% of red fluorences being digestible by RNase and Dnase, respectively.

DAPI Staining Improveo for Quantitative Cytofluorometry
Shinsanichi NAKADA and Setsuya FUJITA
Department of Pathology, Kyoto Prefectural University of Medicine, Kyoto

DAPI (4',6-diamidino-2-phenylinolcic acid) is a fluorescein which binds strongly to DNA molecules with a preference for AT base pairs, exhibiting a high quantum efficiency, so that very small amount of DNAs such as those in viruses, chloroplasts, and mitochondria can be easily visualized with a high S/A ratio. Since the staining procedure of DAPI is very simple and requires no hydrolysis, the staining can be performed under physiological conditions. But there have been unsatisfactory studies on DAPI quantification for cytofluorometry. We investigated DAPI quantification in basic experiments using cell smears and novel films, and developed an optimal staining procedure of DAPI, with some instrumental adjustment, suitable for epifluorescence cytofluorometry. The result is summarized as follows: (1) nonspecific cytoplasmic fluorescence becomes negligible by decreasing a DAPI concentration lower (i.e., to 50 ng/ml) than that usually used, (2) fluorescence quenching is markedly decreased by adding electron donors in the mounting media, (3) errors resulting from light scattering and nano diffration become small by reducing the aperture of an objective lens until halos disappear.

Determinations of Absorption and Fluorescence Spectra of Tissue-bound Dyes by New Microspectrophotometric Apparatus
Tetsuro TAKAMATSU and Setsuya FUJITA
Department of Pathology, Kyoto Prefectural University of Medicine, Kyoto

Absorption and fluorescence spectra of the dyes must be detected directly on smears or histological sections for the purpose of color image analysis and fluorescence histochemistry. This paper reports the new microspectrophotometric apparatus (Olympus) which can automatically and conveniently measure absorption and fluorescence curves in situ.

This system is composed of two subsystems. The optical subsystem consists of a high pressure mercury lamp and tungsten lamp as the light source, fluorescence microscope with epifluorescence (Olympus BH-PH), grating monochromator (H.10, Jobin Yvon) and photomultiplier. The control system has the capacities for controlling the wavelength-scanning of monochromator and the data display operating under the control of microcomputer (HP-97). It also serves the data-analysis including data manipulation, storage and display. Almost specimens could be measured in the spectral ranges from 400 to 700 nm at intervals of 2nm within 1 min.

Some spectral curves of the dyes with weak and rapid fading fluorescence showed a few fluctuations and a shift of the peak. The spectral curves of these dyes were determined at intervals of 5nm.