Mapping Fluorescence Microphotometry System

MapAnalyzer

Bringing new techniques to your research.

In addition to conventional observation and photomicrographic techniques, the quantitative and qualitative analysis of specimen through microphotometry is more and more in demand. In the medical and biological fields, microphotometry is established technique for quantitative analysis of various intercellular substances. MapAnalyzer was developed for quantitative microanalysis of distribution of various substances, such as neurotransmitters and neuromodulators, in the large tissue slice. With Yamato Scientific Co.， fs MapAnalyzer, the possibilities of your research will be extended.

- Application -

(1) Quantitative analysis of immunohistochemical fluorescence
- Distribution of substances in animal and human slices
- Detection of abnormal changes of substances in diseased tissues
- Detection of effect of drugs on the central nervous system and others in the animal experiments
- Elimination of non-specific autofluorescence
- Comparison analysis among various substances in the same slice

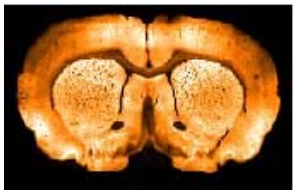
(2) Quantitative analysis of enzyme-labelled immunohistochemistry and various histochemistry

(3) Measurement of fluorescence chelating agent or fluorescence ligand

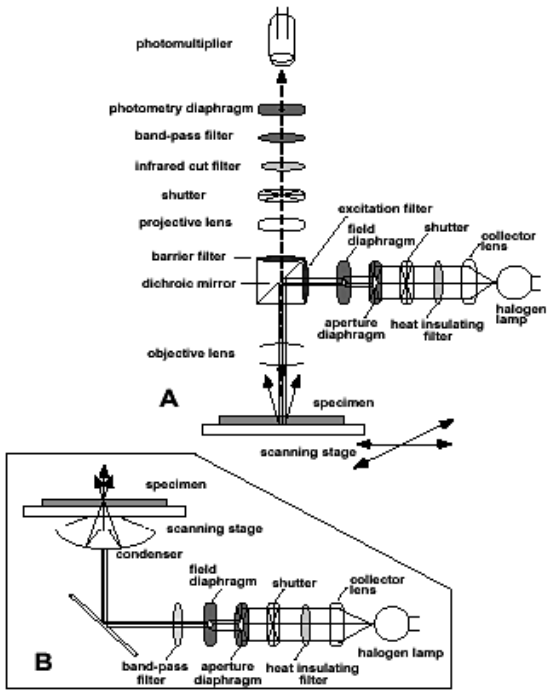
(4) Quantitative analysis of DNA or RNA

(5) Quantitative analysis of autoradiographs or X-ray film

(6) Cytotoxicity testing



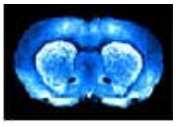
Quantitative immunohistochemical distribution of calmodulin-dependent protein kinase II in the coronal slice of a rat brain.



A second halogen lamp and a band-pass filter of various wavelengths in the visible region are mounted under the scanning stage. Thus, slices stained for histochemical or enzyme-labeled immunohistochemical analysis as well as films such as autoradiographs, can be analyzed quantitatively.

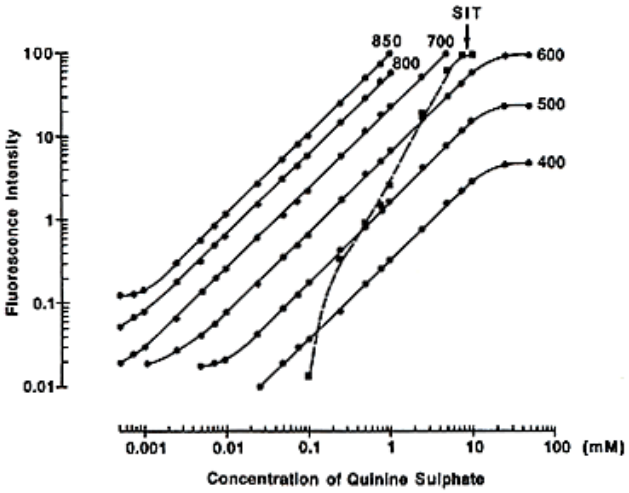
An image of the quantitative distribution of a substance can be obtained as follows: (1) the target substance labeled by immunofluorescent staining in a microarea of a slice is illuminated by a fine excitation beam (the minimum diameter on a slice is 5 µm) which is narrowed by a field diaphragm and aperture diaphragm; (2) the fluorescence in this area is collected into the photometer by the objective lens through a photometry diaphragm, and its intensity is measured; (3) the slice is moved by a two-dimensional scanning stage (the maximum stage motion is 140 mm x 140 mm), and the fluorescence intensity in the next microarea is measured; (4) the measured fluorescence intensity in each microarea is collected in a host computer, where it is analyzed for reconstruction of an image of the entire scanned area; and (5) the image can be viewed as a close-up, in full or at an angle, and displayed quantitatively as a colored or monochromatic image. The actual intensity in a specific region is displayed when that region is selected by a cursor on the image on a TV monitor.

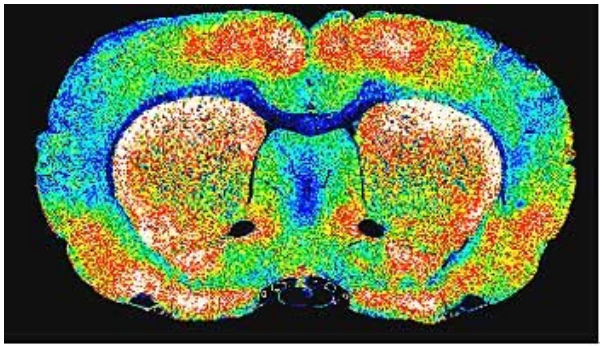
Relationship between fluorescence intensity and quinine sulfate concentration. Fluorescence intensity originating from 0.0005 to 50 mM quinine sulfate in 0.1 N sulfuric acid was finely measured using a MapAnalyzer or image analyzer (Hamamatsu, C1966, Japan) combined with an SIT camara (Hamamatsu, TH9659). Working curves from the MapAnalyzer at each photomultiplier voltage and from the image analyzer are indicated by solid lines (numbers indicate photomultiplier voltage) and a dotted line, respectively. The measurement conditions were as follows: excitation range, 385 to 425 nm; band-pass interference filter, 470 nm; and objective lens, 20x/0.75 (magnification/ numerical aperture).
(See, Folia Pharmacol. Jpn. 91: 173-180, 1988)



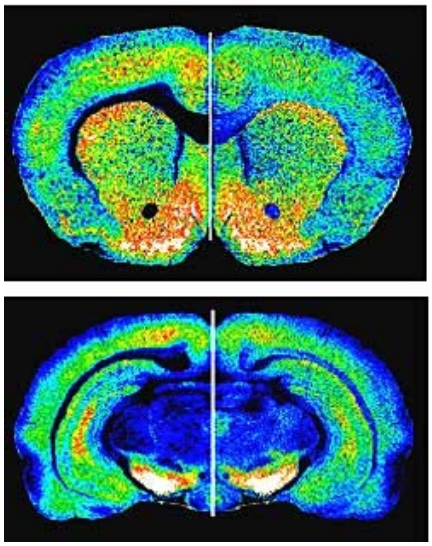
Quantitative immunohistochemical Distribution of tyrosine hydroxylase in the coronal slice of a rat brain.

The quantitative linearity, sensitivity and resolution of MapAnalyzer surpass those of image analyzers used with TV cameras, and the sensitivity, reproducibility and facility of this method are greater than those of the HPLC method. Also, the measuring area of this analyzer is far larger than that of laser confocal microscopes.
(See, J. Neurosci. Methods 85: 161-173, 1998)

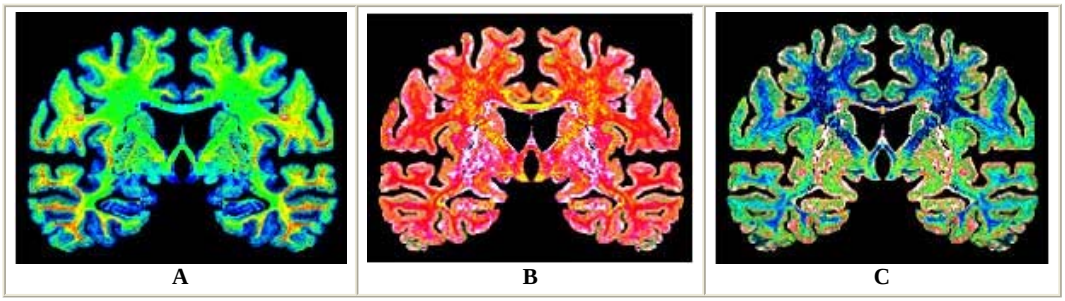




Quantitative immunohistochemical distribution of tyrosine hydroxylase in the coronal slice of a rat brain. The stained slice was measured at 20-µm intervals, and the distribution of the intensity obtained from approximately 250,000 microareas is displayed. Markedly intense tyrosine hydroxylase-like immunoreactivity was distributed in the dorsolateral area of the neostriatum, nucleus accumbens, olfactory tubercle and motor cortex.



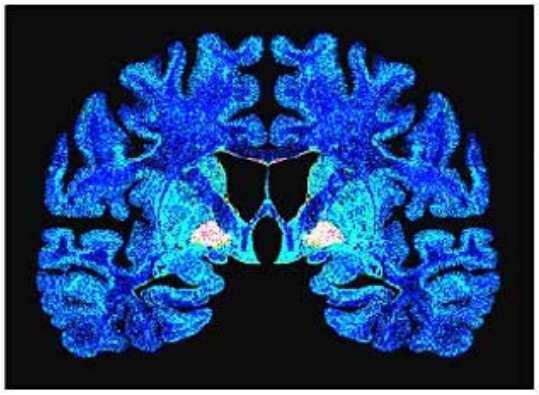
Quantitative immunohistochemical distributions of glutamate decarboxylase (left side) and substance P (right side) in the same slices of a rat brain. Anterior (upper) and posterior (bottom) regions of the brain were analyzed. Double-stained slice was measured at 20-µm intervals. Markedly intense glutamate decarboxylase-like and substance P-like immunoreactivities were distributed in the ventral pallidum and substantia nigra.



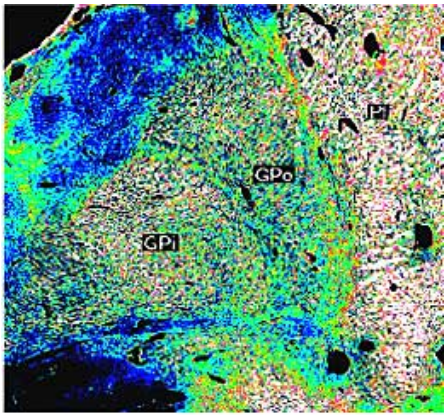
A B C

Elimination of autofluorescence from the human brain slice
A: Nonspecific autofluorescence
B: Immunohistochemical fluorescence superimposed on nonspecific autofluorescence
C: Quantitative immunohistochemical distribution of choline acetyltransferase in the normal human brain slice, i.e., A-B

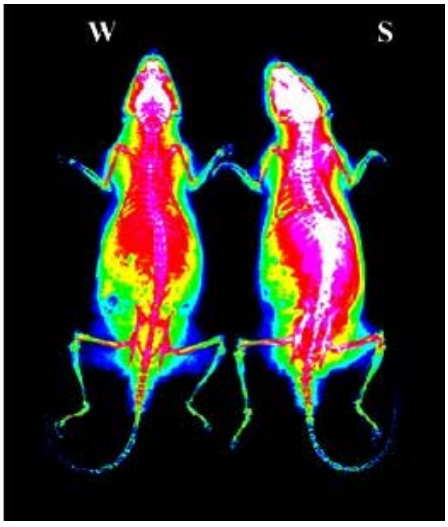
Pure immunohistochemical fluorescence intensities can be obtained automatically from human brains containing various autofluorescences using MapAnalyzer.
Measuring points: approximately six millions at 50-µm intervals.
(See, J. Neurosci. Methods 85: 161-173, 1998)



Quantitative immunohistochemical distribution of substance P in a brain slice of an adult normal human (male, age 50). Data were obtained from approximately six million regions in the brain at 50-µm intervals. Conspicuously intense substance P-like immunoreactivity was observed in the internal segment of the globus pallidus. The immunoreactive intensity in the internal segment of the globus pallidus was approximately twice as high as that in the external segment of the globus pallidus.
(See, Neurosci. Res. 35: 339-346,1999)



Quantitative distribution of Nissl bodies in a human brain slice. The slice was stained with cresyl violet, and the distribution in the globus pallidus and putamen area was analyzed through measurement of the transmission densities. GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; Pt, putamen.
(See, Neurosci. Res. 35: 339-346, 1999)



Quantitative distribution of bone calcification of spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY). X-ray film was reversed and the degree of blackening was measured. A higher level of calcification is observed in the various bones of SHR compared with those of the WKY.
(See, Brain Res. Bull. 30: 107-113, 1993)

-Specification -

Microscope	Epi-fluorescence microscope
Photometry	Fluorescence and Transmittance mode
Measuring spot	Min. diameter: 20 µm on a slice (standard) 5 µm on a slice (option)
Light source	Halogen lamp
Detector	Photomultiplier tube
Scanning stage	Motor-driven X-Y stage Min. stepping movement: 1 µm, Max. stage motion: 140x140 mm Scanning speed: 10 mm/s
Dark box	Desktop type
Computer	OS: Windows NT or Windows 2000
Software	Newly developed software for automatic analysis Save mode: CSV format or BMP file Graphics: two-dimensional and three-dimensional display

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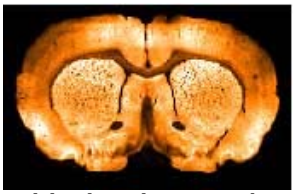

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Tyrosine hydroxylase