Mapping of Inclination of Central Nervous Fiber Tracts in Polarized Light Microscopic Images Using Fuzzy Logic

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ABSTRACT: Nine polarized light microscopic pictures under azimuths from 0° to 80°of the same histological section were digitised, and so nine greyscale pictures were produced. Each pixel was characterised by a set of nine intensities representing different azimuths. This set of intensities was used to draw conclusions about the inclination of the fibers at this point. Therefore a fuzzy procedure was used. The peak intensity was classified by the linguistic variable *intensity* and the number of intensities which are higher than a threshold value was classified with the linguistic variable *peak-width*. The combination of both linguistic variables lead to the classification of each pixel as belonging to the grey or white matter and provided information about the inclination of the fibers in the white matter. Four classes of inclination of the fibers (flat to steep) could be visualised by different colours. The resulting map of fiber inclination corresponded well to anatomical knowledge achieved by confocal laser microscopy.

KEYWORDS: fuzzy logic, imaging, polarization microscopy, central nervous fibers, mapping, human brain, internal capsule, basal ganglia

INTRODUCTION

The structure and anatomy of the cerebral white matter is of great interest, because many neurologic diseases affect the white matter. So many clinical investigations were performed to visualise the architecture of nerve fibers in the cerebral white matter by magnetic resonance tomography (Peled et al. 1998). Nevertheless in a histological preparation an automatic analysis of inclination of nerve fibers is difficult to asses. Our intention was to analyse histological samples regarding to the inclination of the fibers. Therefore polarization microscopy was used.
Polarized light microscopy can selectively visualise anisotropic structures. The normal light can be polarized when it passes through a polarization filter (polarizer). Then the optically polarized light passes through the sample and into a second polarizer (analyzer), that polarizes light in a perpendicular plane with respect to the first polarizer. Birefringence is able to twist some of the light so that it can pass through the analyzer and can be imaged. The birefringence of the nervous tissue has been well known for a long time (Schmidt 1923, 1924, Schmitt 1936, Kretschmann 1967, Wolman 1975). Because the presence of anisotropy indicates polarity and order, polarization microscopy can be used to visualise long fiber tracts in the brain (Fraher et al. 1970, Miklossy et al. 1991). The orientation of the fibers influences the transmission of plane-polarized light at different velocities at different azimuths.
Especially parallel, horizontally cut fibers give a bright signal at a special azimuth whereas the signal decreases after rotation of 45°. That means that there is a peak of brightness under rotation of the polars. The steeper these fiber tracts are, the less bright this peak is. If the fibers are cut transversely then there is no peak, and the brightness does not change under rotation. Grey substance does not give any signal of light. Thus different fiber tracts of different orientation and order produce areas of different brightness.
The information about fiber inclination is contained in the sequence of all pictures produced under rotation of the polars or under rotation of the sample. Our intention was to find a method to interpret this sequence automatically and draw a map of the fiber inclination in the sample. Because the different intensities can be linguistically characterised as dark to bright, a procedure using fuzzy logic was obvious (Zimmermann 1996, Tizhoosh 1998).

METHODS

Four human cadaver brains were fixed in 4% formalin solution. Three brains were cut horizontally, parallel to the line between anterior and posterior commissure (ACPC). The other brain was cut frontally. Our main interest was focused on the internal capsule and on the basal ganglia, all deep cerebral structures of high clinical importance. The samples were sectioned on a freezing microtome at 60 µm. The sections were serially collected and coverslipped without staining procedure.

The slices were placed between two rotatable crossed polarizing filters and illuminated with light. Digitalisation of the slices were performed with the monochrome camera Sony XC 75-CE which was connected to a personal computer equipped with a frame grabber card. The software used was OPTIMAS 4.10. This way greyscale pictures could be achieved. The crossed polars can be rotated without changing the orientation of the sample. So homologous pixels in the pictures under different rotation angles of the polars belong to the same point in the sample.

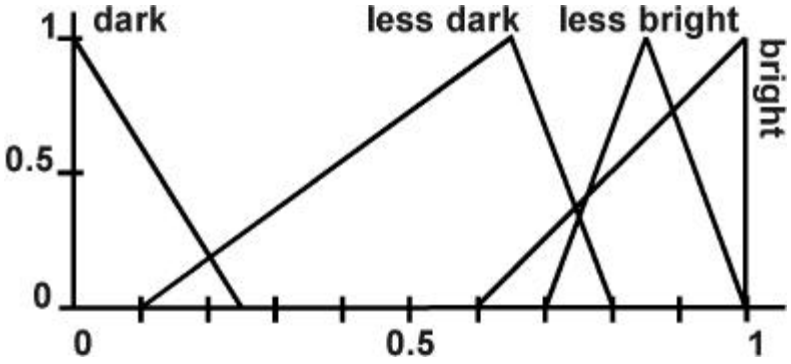

Figure 1: The linguistic variable *intensity.*

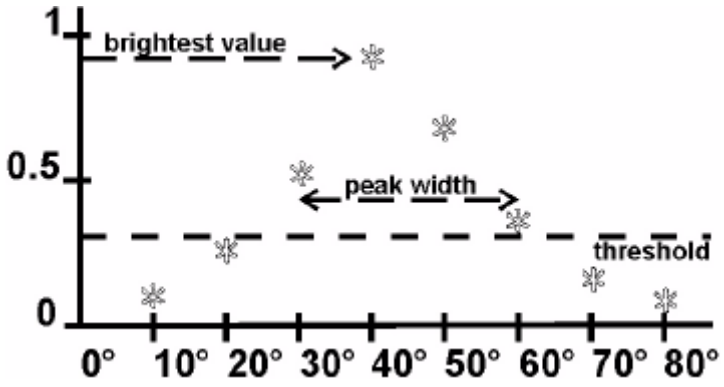

Figure 2: Two important parameters: peak intensity and peak width.

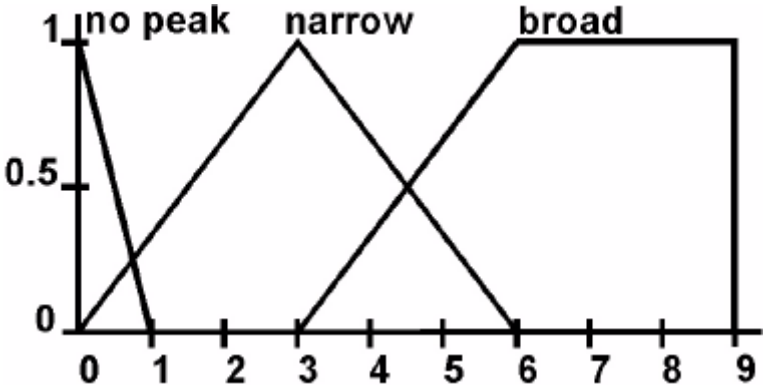

Figure 3: The linguistic variable *peak-width.*

Each sample produced nine different pictures under nine rotation angles (azimuths) from 0° to 80°. Thus a set of nine values of intensity under the different azimuths was assigned to each point in the sample. The greyscale intensities range from 0 to 255 and at first these intensities were normalised by division through 255.

$$A_{x,y} = \{ \text{intensity}_{x,y}(0°), \text{intensity}_{x,y}(10°), .. , \text{intensity}_{x,y}(80°) \}$$

The nine values of intensity contain information about the orientation of the nerve fibers at this point. So an adequate analysis should show if a pixel belongs to the grey or to the white cerebral matter. Moreover it should provide information about the inclination of the fibers in the white matter.
The linguistic variable *intensity* was defined to characterise the intensity of the pixels (Fig. 1). Four different classifications of intensity were named as dark, less dark, less bright and bright.
The first aim was to find the regions which belong to the cerebral grey matter. This classification can be done very easily, because all nine intensities have to be dark. So the rule is defined as

$$\mu_{grey} = \text{dark}(0°) \text{ and } \text{dark}(10°) \text{ and } .. \text{ and } \text{dark}(80°) = \min (\mu_{dark0°}, \mu_{dark10°}, .. , \mu_{dark80°}).$$

The second aim was to classify the inclination of the fibers in the white matter. The most important information in the nine intensity values are contained in two parameters: One parameter should be the brightest intensity. The peak intensity will be higher the more flat and will be less the more steep the fibers run. On the other hand the flatter the fibers are the more accentuated the peak will be. So another interesting parameter should be the width of the peak of the intensities measured above a threshold value (Fig. 2). This second parameter is defined as the number of intensity values which are higher than the threshold value (in this case 0.4). The number can only reach between 0 and 9 and the linguistic variable *peak-width* was defined to classify the peak width (Fig. 3) as no peak, narrow and broad.
A truth table was defined to classify the inclination of the fibers according to these two linguistic variables (table I). The table contains such rules like: if the highest intensity is bright and the peak width is narrow then the inclination of the fibers have to be flat. This rule can be processed as $\mu_{flat} = \min (\mu_{bright}, \mu_{narrow})$.
Defuzzification will be done by searching the rule with the highest membership function and the pixel will be classified as flat, less flat, less steep or steep which can be visualised as different colours (or greyscales).

peak width/intensity	dark	less dark	less bright	bright
no peak	no fibers	steep	less steep	artefact
narrow	no fibers	less steep	less flat	flat
broad	artefact	less steep	less flat	flat

Table I: Truth table

RESULTS

Fig. 4 shows some representative results. A-D show horizontal sections through the internal capsule and E-H show frontal sections through the internal capsule. Fig. 4 B and F give an anatomical overview over the structures which are shown in the other pictures and Fig. 4 A and E are examples of one of the original nine polarization images.
In Fig. 4 D and H show a map of μ_{grey} and the grades of membership of the pixels are represented as three greyscales. Thalamus, nucleus caudatus , putamen and pallidum are classified correctly. Grey substance with high content of fibers (as thalamus and pallidum) achieve smaller grades of membership as grey substance with less content of nervous fibers (as nucleus caudatus and putamen). Moreover the lateral nuclear group of the thalamus contains a lot of bundles of fibers which run in direction to the internal capsule and these bundles are definitely shown as dark bands in the map.
Fig. 4 C and G represent the maps of the inclination of the fiber tracts. In the horizontal cut through the internal capsule this procedure distinguished different fiber tracts. In the crus anterior of the internal capsule two fiber tracts can be distinguished. One fiber system contains fibers which are classified as flat and less flat and which run horizontally into the thalamus. This fiber system is known as the anterior thalamic peduncle. A second fiber system is somewhat smaller and arranged in small bands. It was classified as steep and less steep. These fibers run from the frontal lobes to the brain stem and belong to the frontopontine tract. In the crus posterior also two different fiber

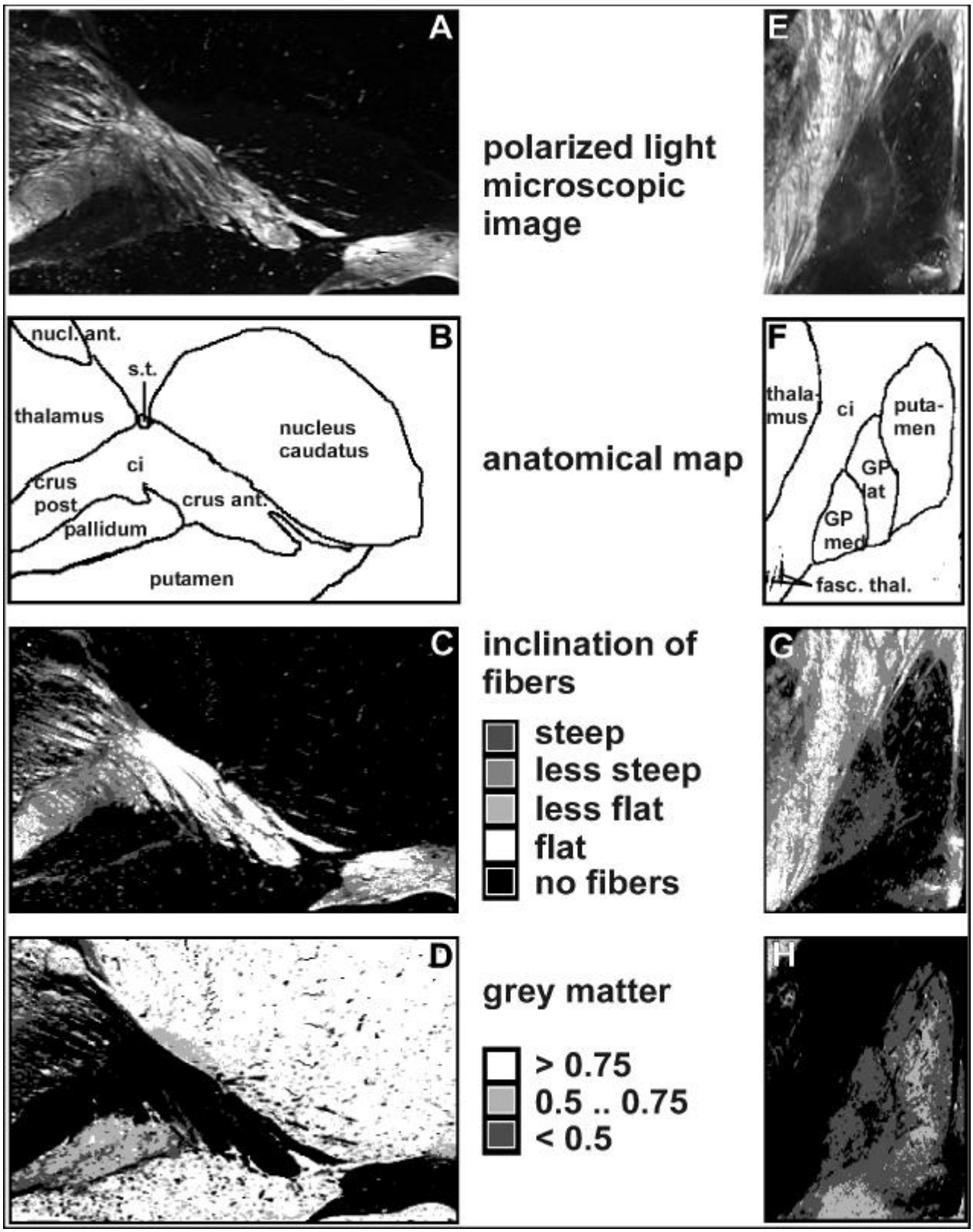

Figure 4: A-D: horizontal section parallel to ACPC-line. E-H: frontal section of the internal capsule. A and E: examples of one of the original polarization images; B and F: anatomical maps of this region; C and G: maps of the inclination of the fibers; D and H: maps of the classification as grey substance; Abbreviations are: nucl. ant. = nucleus anterior of the thalamus, s.t. = stria terminalis, ci = capsula interna, crus ant. = crus anterior, GP lat = pars lateralis of the globus pallidum, GP med = pars medialis of the globus pallidum, fasc. thal. = fasciculus thalamicus.

systems were correctly visualised. Horizontally fibers from the thalamus were classified as flat and less flat, and steeper fibers to the spinal cord and the brain stem, which belong to the pyramidal tract, were classified as steep and less steep. In the edge between nucleus caudatus and thalamus there runs a separate fiber tract (stria terminalis), and consists of transversely cut fibers. Accordingly these fibers were classified as steep.
In the frontal cut (Fig.4 G) different fiber tracts in the internal capsule could be detected, too. Fibers of the pyramidal tract are now cut horizontally and these are correctly classified as flat. Fibers from the thalamus are cut more transversely than the pyramidal tract fibers and are classified as less steep. At the bottom of the picture in the internal capsule the fasciculus thalamicus can be seen.

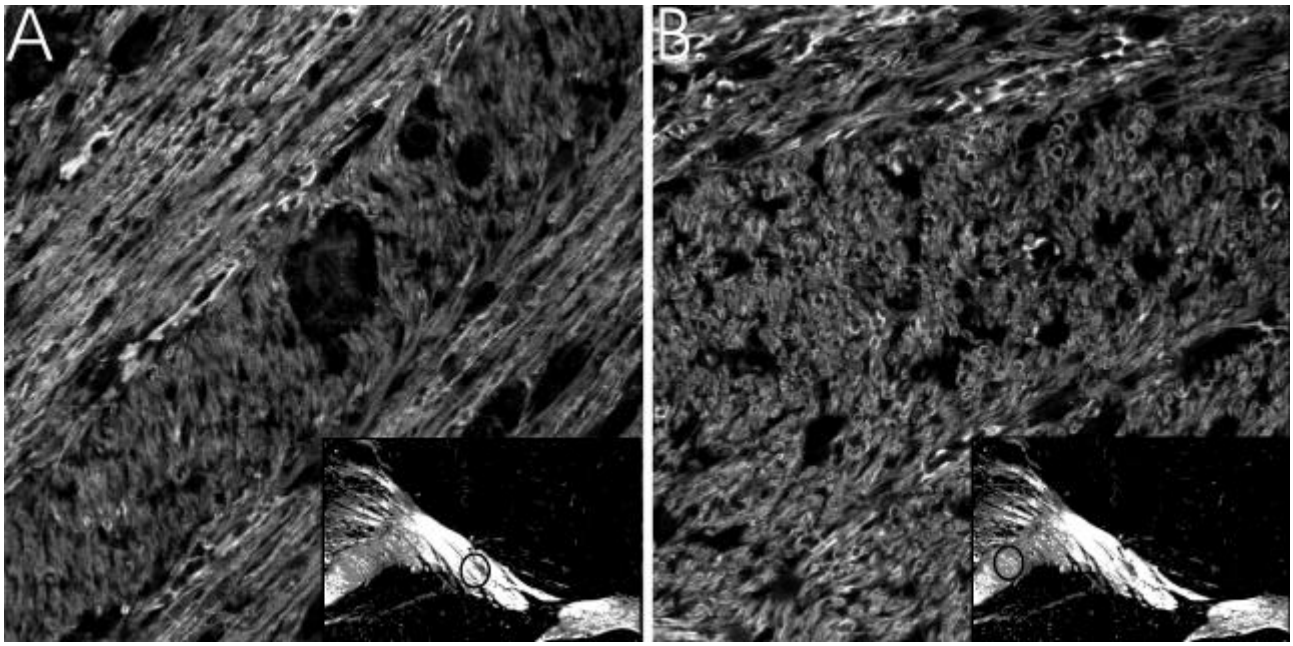

Figure 5: Evaluation of the results with confocal laser microscopy. The place of these pictures in the map of fiber inclination is shown in the right lower corner. A. The frontopontine tract fibers in the middle of the picture and fibers of the pedunculus thalami anterior with horizontally cut fibers can be distinguished in the crus anterior of the internal capsule. B Pyramidal tract fibers are cut transversely and are traversed by horizontally cut fiber bundles of the pedunculus thalami superior.

DISCUSSION

The combination of nine polarization pictures of one sample under different azimuths allows to draw conclusions about the inclination of the fiber tracts. The results achieved by fuzzy methods correspond well to the known anatomical structure of this region. Moreover we evaluated the correctness of these results with confocal laser microscopy. Nervous fibers can be labelled with the fluorescence marker DiI (1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate). This way the fiber architecture of these regions was visualised in detail (Fig. 5). Nevertheless this confocal procedure is very time consuming. To achieve a map to overview the central nervous fiber tracts a method is needed which is fast and easily applicable. The presented fuzzy procedure could be one possibility to do this.
The resulting maps of fiber inclinations may be used in different ways. One interesting possibility is to use the classification of pixels according to fiber inclination for morphometrical purposes. It is a procedure to distinguish different fiber tract systems and may be used to calculate values of area which are occupied by the different fiber tracts. Another possibility may be to map serial slices of the brain in order to perform threedimensional reconstructions of these fiber tracts. This approach would lead to 3D-anatomic atlasses. We could demonstrate that the electrical impedance in brain tissue is dependent on fiber myelinisation and orientation of the fibers (Axer et al. 1998, 1999). Such an anatomical 3D-atlas would be of great importance to compare tissue impedances and the fiber structure at this point.

REFERENCES

Axer, H.; Stegelmeyer, J.; Keyserlingk, D.G.v., 1998, "Semiquantitative Analysis of Myelin Content in Human Central Nervous Tissue With Methods of Fuzzy Geometry." Proc. 6th European Congress of Intelligent Techniques and Soft Computing, EUFIT 1998, Aachen, Germany, pp. 1827-31.

Axer, H.; Stegelmeyer, J.; Keyserlingk, D.G.v., 1999, "Comparison of tissue impedance measurements with nerve fiber architecture in human telencephalon: value in identification of intact subcortical structures." J Neurosurg 90, pp 902-909.

Fraher, J.P.; MacConaill, M.A., 1970, "Fibre bundles in the CNS revealed by polarized light." J Anat 106, p. 170.

Kretschmann, H.-J., 1967, "On the demonstration of myelinated nerve fibers by polarized light without extinction effects." J Hirnforsch 9, pp. 571-575.

Miklossy, J.; Van der Loos, H., 1991, "The long-distance effects of brain lesions: Visualization of myelinated pathways in the human brain using polarizing and fluorescence microscopy." J Neuropath Exp Neurol 50, pp.1-15.

Peled, S.; Gudbjartsson, H.; Westin, C.-F.; Kikinis, R.; Jolesz, F.A., 1998, "Magnetic resonance imaging shows orientation and asymmetry of white matter fiber tracts." Brain Res 780, pp. 27-33.

Schmidt, W.J., 1923, "Zur Doppelbrechung des Nervenmarks." Zeitschr f wiss Mikroskopie 41, pp. 29-38.

Schmidt, W.J., 1924, "Die Bausteine des Tierkörpers im polarisierten Lichte." Bonn/Cohen, Germany.

Schmitt, F.O.; Bear, R.S., 1936, "The optical properties of vertebrate nerve axons as related to fiber size." J Cell Comp Physiol 9, pp. 261-273.

Tizhoosh, H. R., 1998, "Fuzzy Bildverarbeitung." Berlin/Springer, Germany.

Wolman, M., 1975, "Polarized light microscopy as a tool of diagnostic pathology. A review." J Histochem Cytochem 23, pp. 21-50.

Zimmermann; H.J., 1996, "Fuzzy Set Theory." Boston/Kluver Acad. Publ., USA.