ABSTRACT
Recently, many attractive brain-computer interface (BCI) and brain-machine interface (BMI) have been proposed. It is necessary to predict activity of a neuronal network in the brain to control an outer equipment by BCI and BMI exactly. However, it is difficult for the action potential to identify from the input-output data because a neuronal network in the brain is multilayered structure, and the multilayer structure is too complex. Therefore we should discuss an analysis model of action potentials using a simple structure of neuronal network more like a culture dish. In this paper, we propose a model to analyse logicality of signals and connectivity of electrodes in a culture dish. In addition, we introduce a “fuzzy bio-indicator” to show activity in a culture dish of rat hippocampal neurons. We show here the usefulness of fuzzy bio-indicator through numerical examples and action potential detected from the culture neuronal network.

General Terms
Living Neuronal Network, Culture System, Fuzzy Connectives

1. INTRODUCTION
Recently, many papers of brain-computer interface (BCI) and brain-machine interface (BMI) have been published [1, 2]. An outer computer and machine are controlled by discriminant models using brain action potentials detected through a device such as near-infrared spectroscopy (NIRS) and electroencephalograph (EEG). However, it is difficult for the action potential to identify from the input-output data because a neuronal network in the brain is multilayered structure, and the multilayer structure is too complex. Therefore we should discuss an analysis model of action potentials using a simple structure of neuronal network more like a culture dish. In this paper, we propose a model to analyse logicality of signals and connectivity of electrodes in a culture dish. In addition, we introduce a “fuzzy bio-indicator” to show activity in a culture dish of rat hippocampal neurons. We show here the usefulness of fuzzy bio-indicator through numerical examples and action potential detected from the culture neuronal network.

In this paper, we propose a model to analyse logicality of signals and connectivity of electrodes in a culture dish of rat hippocampal neurons [14, 15]. In addition, we introduce a “fuzzy bio-indicator” to show activity in a culture dish. This indicator is a kind of mapping methods to show logicality and connectivity of pulse frequency from active potential of neuronal network. We’re not sure that that system is unique, however, we discuss here how to indicate the logicality and the connectivity of living neuronal network. Rat hippocampal neurons are organized into complex networks in a culture dish with 64 planar microelectrodes. A multi-site recording system for extracellular action potentials is used in order to record their activities in living neuronal networks and to supply stimulus input from the outer world to the vitro living neural networks. The living neuronal networks are able to express several patterns independently, and such patterns represent fundamental mechanisms for intelligent information processing [16, 17].

We follow the works of Bettencourt et al. [18] such that they classify the logicality and the connectivity of action potentials of three electrodes on the multi-site recording system according to their entropies and have discussed the characteristic of each classification. However, they only discuss the static aspects of logicality and connectivity relations among the electrodes but not the dynamics of such connectivity concerning how the strength of electrode connection changes when a spike is fired. To address this issue, first, we propose a new algorithm using parametric fuzzy connectives, that consist of $t_{-norm}$ and $t_{-conorm}$ operators [19, 20], in order to analyse the logicality and the connectivity of those three electrodes. Next, we classify propagation patterns of pulse frequency to three formulations, which are transmission, diffusion, and absorption, and we define an evaluation of fuzzy inclusion degree as corresponding of pulse frequency between electrodes for each propagation pattern. Finally, we plot the activity of pulse frequency in figures as the fuzzy bio-indicator. As a result, the logicality of neuronal network in the culture dish is described with fuzzy connectives that consist of $t_{-norm}$ and $t_{-conorm}$, and the connectivity is described with fuzzy inclusion degree for each propagation pattern. We show here the usefulness of fuzzy bio-indicator through numerical examples and action potential detected from culture neuronal network.

2. NEURON CULTURE AND MULTIELECTRODE ARRAY
The conduct of all experimental procedures was governed by The Animal Welfare, Care and Use Committee in AIST. The hippocampus neurons were prepared from a Wistar rat on embryonic day 17-18 (E17-18) and cultured by the previously described method [16]. Briefly, neurons were dissociated by treatment with 0.175%
t sum, algebraic sum, bounded sum and drastic sum. The product, bounded product and drastic product. The medium is based on D-MEM/F12, supplemented with fetal serum (Gibco, U.S.A.) and trypsin (Gibco, U.S.A.) and cultured by plating 500,000 cells in a 7 mm diameter-glass ring on poly-D-lysine coated MED probe (Alpha MED Sciences, Japan), which has 64 planar placed microelectrodes. The field action potentials were recorded 10-100 days after the start of the culture. The spontaneous action potentials (sAPs) were gathered with the MED64 system at a 20 kHz sampling rate. All experiments were carried out at room temperature (20 ± 2 °C). The recorded spikes were detected by our developing program, sorted and classified by the amplitude versus decay time distributions using k-means cluster cutting method and converted to event trains.

3. LOGICALITY OF NEURONAL NETWORK
Fuzzy connective operators consist of t − norm and t − conorm operators. The t − norm T is a projection function expressed by
\[ T(x, y) = \begin{cases} 0 & \text{if } (1 - x) p_n + (1 - y) p_n - (1 - x) (1 - y) p_n - (1 - x) (1 - y) p_n \leq 0 \\ 1 & \text{otherwise} \end{cases} \]

and the drastic operator, which is a dual function of the norm operator, which is expressed by
\[ S(x, y) = \begin{cases} (x p_c + y p_c - x p_c y p_c)^{1/p_c} & \text{if } (1 - x) p_n + (1 - y) p_n - (1 - x) (1 - y) p_n - (1 - x) (1 - y) p_n \leq 0 \\ 0 & \text{otherwise} \end{cases} \]

where, \( p_n \) and \( p_c \) are parameters.

By changing the values of the parameter \( p_n \) and \( p_c \), the Schweizer operator, \( t − norm \) and \( t − conorm \), express logical operator \( (p_n = p_c = \infty) \), algebraic operator \( (p_n = p_c = 1) \) and drastic operator \( (p_n = p_c = 0) \).

By the Schweizer operator, \( t − norm \), and \( t − conorm \), we formulate a new algorithm for analysing the logicality and the connectivity of the living neuronal networks. Now, we selected an arbitrary set of three electrodes \( x, y, z \), and analysed a coherence pattern between three electrodes. First, we distribute a data set of pulse-time series in several time-bins, and define a time-delay deviation between time-bins of two electrodes. The proposed algorithm is shown in Figure 1. For the electrode z, we shape a fuzzy set of the pulse frequency, \( F_r^z \), at the i-th time-bin by the following membership function which has the center \( a_i^z \) and the width \( c_i^z \).

\[ a_i^z = \left( \frac{q_i^z - lq_i^z}{hq_i^z - lq_i^z} \right) \frac{hq_i^z - lq_i^z}{q_i^z - lq_i^z} \quad (3) \]

\[ c_i^z = \left( E(a_i^z) \right) \quad (4) \]

where, \( q_i^z \) is the number of pulse at the i-th time-bin, \( lq_i^z \) and \( hq_i^z \) are the minimum and maximum number of \( q_i^z \), respectively. \( E(a_i^z) \) is the average value of \( a_i^z \).

Figure 1: Algorithm for Analysis of Action Potentials in Cultured Neuronal Network
The membership function \( F^x_{e,y} \) with the delay deviation \( a_x \) of the electrode \( x \) is shaped as same as the electrode \( z \). Our purpose is to let the degree of coincidence, \( \mu^x_{e,z} \), between \( F^x_{e} \) and \( F^z_{e} \) maximize in the parametric conditions of the electrode \( x \). To let the degree of coincidence maximize, the width of time-bin \( w_x \) and the delay deviation \( a_x \) are changed widely.

\[
\mu_{e,z} = \sup_t \mu F^x(t) \land \mu F^z(t) \tag{5}
\]

\[
\mu_{e,z} = \max_{w_x, a_x} \mu_{e,z}. \tag{6}
\]

We calculate \( \mu_{e,z} \) between the electrode \( y \) and the electrode \( z \) as same as the electrode \( x \) and the electrode \( z \). By obtaining two couples of coincidence degrees, \( \mu_{e,z} \) and \( \mu_{e,z} \), the connection of electrodes is figured as a kind of connectivities in Figures 2.

A : Confluence  B : Ramification  C : Relay

Figure 2: Connectivity of Electrodes

Next, we calculate the output of the Schweizer operator with two centers of membership functions, \( a^x_{i-e} \) of the electrode \( x \) and \( a^y_{i-e} \) of the electrode \( y \).

\[
\begin{align*}
T(a^x_{i-e}, a^y_{i-e}) &= 1 - ((1 - a^x_{i-e}) p_n + (1 - a^y_{i-e}) p_n) \\
S(a^x_{i-e}, a^y_{i-e}) &= ((a^x_{i-e}) p_n + (a^y_{i-e}) p_n - 1) / p_n \tag{7}
\end{align*}
\]

We minimize the error deviation between the center \( a^x_{i} \), and the Schweizer’s output, \( T(a^x_{i-e}, a^y_{i-e}) \) and \( S(a^x_{i-e}, a^y_{i-e}) \), by changing the parameter \( p_n \) of \( t-norm \) and \( p_e \) of \( t-conorm \).

\[
p^* = \{ p_n, p_e \} \min_{p_n, p_e} | T(a^x_{i-e}, a^y_{i-e}) - a^x_{i} |, \tag{8}
\]

\[
S(a^x_{i-e}, a^y_{i-e}) - a^x_{i} \}. \tag{9}
\]

The suitable parameter \( p^* \) represents the logicality of three electrodes. To illustrate the proposed algorithm, we show a simple numerical example. The spike frequency of three examples of electrodes \( x \) and \( z \) are shown in Figures 3 to 5, and Table 1. At each example, we search a time-bin of electrode \( x \) which coincides most with the spike frequency of the sixth time-bin of electrode \( z \). At the first example of However, the "normalized order" of the horizontal axis in each figure normalized the number of spike frequency within each time bin as the same size. Figure 3, the spike frequency "2" of the fourth time-bin of electrode \( x \) coincided most with the spike frequency "2" of the sixth time-bin of electrode \( z \) with the degree \( \mu_{e,z} = 1.0 \) of fuzzy sets. At the second and third examples of Figure 4 and Figure 5 respectively, the spike frequency "3" of the ninth time-bin of electrode \( x \) coincided most with the spike frequency "2" of the electrode \( z \) with \( \mu_{e,z} = 1.0 \) as shown in Figure 4, and the spike frequency "1" of the sixth time-bin of electrode \( x \) coincided most with the the spike frequency "1" of the sixth time-bin of electrode \( z \) with \( \mu_{e,z} = 0.44 \) as shown in Figure 5. We should notice that these results are understandable intuitively.

<table>
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<tr>
<th>Time Bin</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tr>
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<td>0.67</td>
<td>0.0</td>
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<td>0.33</td>
<td>1.0</td>
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<td></td>
</tr>
<tr>
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<td>0.4</td>
<td>0.07</td>
<td>0.07</td>
<td>0.6</td>
<td>0.27</td>
<td>0.4</td>
<td>0.27</td>
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<tr>
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<td>WotZ</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µ</td>
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<td>1.0</td>
<td>1.0</td>
<td>0.36</td>
<td>0.52</td>
<td>0.6</td>
<td>0.52</td>
<td>0.6</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Example 2

| X        | 2 | 3 | 2 | 2 | 0 | 1 | 1 | 3 | 1 | 1  |
| Z        | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0  |
| CoX      | 0.67 | 1.0 | 0.67 | 0.0 | 0.33 | 0.0 | 0.33 | 1.0 | 0.33 |
| WotX     | 0.23 | 0.57 | 0.23 | 0.43 | 0.43 | 0.43 | 0.1 | 0.43 | 0.1 | 0.57 |
| CoZ      | 0.33 |
| WotZ     | 0.1  |
| µ         | 0.68 | 1.0 | 0.68 | 0.19 | 0.19 | 0.26 | 0.19 | 0.26 | 1.0 | 0.26 |

Example 3

| X        | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0  |
| Z        | 0 | 0 | 1 | 2 | 3 | 1 | 0 | 0 | 0 | 0  |
| CoX      | 0.0 | 0.5 | 0.0 | 0.1 | 0.0 | 0.5 | 0.0 | 0.1 | 0.0 | 0.0 |
| WotX     | 0.3 | 0.2 | 0.3 | 0.7 | 0.3 | 0.3 | 0.7 | 0.3 | 0.3 |
| CoZ      | 0.33 |
| WotZ     | 0.1  |
| µ         | 0.17 | 0.44 | 0.17 | 0.17 | 0.17 | 0.44 | 0.17 | 0.17 | 0.17 |

1. \((x, y, z) = (51el, 59el, 60el)\)
2. \((x, y, z) = (43el, 50el, 60el)\)
3. \((x, y, z) = (35el, 42el, 60el)\)
Figure 3: Fuzzy Sets of the First Example of Electrode Analysis

Figure 4: Fuzzy Sets of the Second Example of Electrode Analysis

Figure 5: Fuzzy Sets of the Third Example of Electrode Analysis

Figure 6: Experiments

Figure 7: Analysis of Action Potentials
The result is shown in Figure 7. At the first combination of the electrodes \((51el, 59el, 60el)\), the maximum degrees of coincidence were obtained as \(\mu_{xz} = 0.85\), \(\mu_{yz} = 0.75\) with \(w_x = 11s\), \(w_y = 10s\), and the parameter of Schweizer operator was converged to \(p^* = p_c = 730.5\). At the second combination of the electrodes \((43el, 50el, 60el)\), the maximum degrees of coincidence were obtained as \(\mu_{xz} = 1.0\), \(\mu_{yz} = 1.0\) with \(w_x = 11s\), \(w_y = 10s\), and the parameter of Schweizer operator was converged to \(p^* = p_c = 617.98\). At the third combination of the electrodes \((35el, 42el, 60el)\), the maximum degrees of coincidence were obtained as \(\mu_{xz} = 0.76\), \(\mu_{yz} = 0.91\) with \(w_x = 11s\), \(w_y = 10s\), and the parameter of Schweizer operator was converged to \(p^* = p_c = 630.23\).

From these results, we notice that the pulse fired at 60el at 102.4s propagates to \((51el, 59el) \rightarrow (43el, 50el) \rightarrow (35el, 42el)\), and then, the parameters of Schweizer operator have been converged to infinity, \(p^* = p_c = 730.5\) at \((51el, 59el)\), \(p^* = p_c = 617.98\) at \((43el, 50el)\), and \(p^* = p_c = 630.23\) at \((35el, 42el)\). These parameters mean logical sum. However, we should notice that the parameter of Schweizer operator at around 102.4s is \(p^* = p_c = 0.0\), which means the drastic product. Given this result, we conclude that the logicality of signals among the electrodes was shifted to the logical sum from the drastic product. Consequently, the logicality of signals among electrodes drastically changes from the strong \(AND\)-relation to the weak \(OR\)-relation when a crowd of the pulses was fired.

4. CONNECTIVITY OF NEURONAL NETWORK

In order to discuss the connectivity of living neuronal network, we define propagation patterns of pulse fired at a electrode. We define an inclusion degree of fuzzy numbers as the connectivity of pulse frequency between electrodes for each propagation pattern. Thus, a characteristic of pulse frequency of living neuronal network is configured with logicality of \(\text{norm}\) and \(\text{conorm}\), and connectivity of this fuzzy numerical inclusion degree.

A propagation of the pulse frequency between electrodes is defined with three kinds of patterns, which are transmission, diffusion, and absorption as shown in Figure 8. The transmission shows a pattern that the pulse propagates to some electrode, and the diffusion shows a pattern that the pulse propagates with spread directions.

For each propagation pattern, the connectivity of neuronal network is discussed with an inclusion degree of fuzzy numbers between electrodes. Figure 9 shows the inclusion degree of fuzzy numbers. The fuzzy inclusion degree means how degree of a fuzzy number of the pulse frequency is included by other pulse frequency. Now, the width of fuzzy number \(X, Y\), and \(Z\) is described by \(D_X, D_Y\), and \(D_Z\) respectively. A fuzzy inclusion degree \(\gamma\) of fuzzy number \(Z\) with fuzzy number \(X\) and \(Y\) is defined as follows:

\[
\gamma = \frac{D_X}{D_Z} \times \frac{D_Y}{D_Z}
\]

where, the degree \(\gamma\) get closer to one when the inclusion level of fuzzy numbers is high, and then the connectivity between electrodes is tight.

5. INDICATOR OF LOGICALITY AND CONNECTIVITY

To indicate the logicality and connectivity of neuronal network, we should discuss delay time of pulse propagating to a neighbor electrode from a certain electrode. Since the propagation speed of a pulse is approximately 100m/s, and distance between electrodes is 450um, the delay time between electrodes is 0.0045ms. However, we estimated propagation time between electrodes at 10ms because the connection of neuronal network is not straight, and chemical neurotransmitter and synapse delay at the synapse have to be considered. In addition to parameters for analysing action potential, sampling frequency of the MED64 is 20kHz, and the time-bin is obtained at 4s in consideration of the overlap of time data. The number of time-bins is 30 because the measurement time is 120s.
as follows;

\[ a : (19el, 44s - 48s) \]
\[ b : (3el, 88s - 92s), (4el, 88s - 92s), (11el, 88s - 92s) \]
\[ c : (31el, 88s - 92s) \]
\[ d : (39el, 80s - 84s) \]

Figure 10: Transmission of Pulse Frequency

Figure 10 shows a result of transmission of pulse frequency. The horizontal axis expresses value of parameter \( p_n \) and \( p_c \) of fuzzy connectives, and the vertical axis shows the inclusion degree of fuzzy numbers. The 14 most suitable parameters exist near \( \gamma = 0.0 \) and \( p_n = 1.0 \), and 75 parameters exist near \( \gamma = 1.29 \) and \( p_c = 430.0 \). In addition, the suitable parameters are distributed near \( \gamma = 2.45 \) and \( p_c = 999.9 \), \( \gamma = 3.12 \) and \( p_c = 999.9 \), \( \gamma = 3.85 \) and \( p_n = 508.0 \), and \( \gamma = 3.97 \) and \( p_c = 508.0 \). As a result, the most suitable parameters are located at \( \gamma = 1.29 \) and \( p_c = 430.0 \). We should notice that the fuzzy connective operator is adjusted to a weak OR because the parameter \( p_n = 10.7 \) means logical product.

Figure 11: Narrow Diffusion and Absorption of Pulse Frequency

Figure 11 shows a result of diffusion-with-narrow-direction, and absorption of pulse frequency. The most suitable parameters exist near \( \gamma = 1.72 \) and \( p_n = 10.7 \), \( \gamma = 2.33 \) and \( p_c = 30.0 \), and \( \gamma = 3.79 \) and \( p_c = 37.7 \). As a result, the most suitable parameters are located at \( \gamma = 1.72 \) and \( p_n = 10.7 \). We should notice that the fuzzy connective operator is adjusted to a weak AND because the parameter \( p_n = 10.7 \) means logical product.

Figure 12: Wide Diffusion of Pulse Frequency

Figure 12 shows a result of diffusion-with-wide-direction of pulse frequency. The most suitable parameters are located near the large inclusion degree. The 9 parameters are distributed near \( p_c = 15.2 \), 14 parameters are distributed near \( p_c = 30.0 \), 4 parameters exist near \( p_c = 35.5 \), 18 exist near \( p_c = 53.7 \) and \( p_c = 206.6 \), 26 exist near \( p_c = 349.8 \), 104 exist near \( p_c = 630.3 \), and 4 exist near \( p_c = 5.2 \) and \( p_n = 397.5 \). In particular, the most suitable parameter is located at \( p_c = 630.3 \). We should notice that the fuzzy connective operator is adjusted to a weak OR because the parameter \( p_c = 630.3 \) means logical sum.

Figure 13: Whole Diffusion of Pulse Frequency

Figure 13 shows a result of diffusion-with-whole-direction of pulse frequency. The suitable parameter spreads widely. As a result, the fuzzy connective operator does not converge the specific values. That means that various kinds of logicality are mixed when the pulse frequency spreads in all directions.

From the overall result, we notice that the inclusion degree becomes higher and various kinds of operators such as logical sum
and logical product appear when the diffusion range is wide. In other words, the pulse is sure propagating without a loss of frequency when the propagation spreads with various kinds of operators widely.

6. CONCLUSION
In this paper, we discussed how to indicate the logicality and the connectivity of living neuronal network with fuzzy connective operators and fuzzy inclusion degree. We should analyse the relationship between pulse of living neuronal networks and propagation pattern more deeply in the near future.

7. ACKNOWLEDGMENTS
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8. REFERENCES


