Optical flow analysis to classify activity patterns in living neuronal networks

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Abstract: Neuronal cultures provide an accessible way to observe and model the behavior of living neuronal networks. In this study, spontaneous activity recorded in such neuronal cultures is analyzed with the aim to find distinct spatiotemporal patterns of spontaneous activity, such as synchrony or spiral fronts. Human and rat neurons, cultured on flat or engineered surfaces, are analyzed along different days *in vitro* (DIV) using Neuropatt, a MATLAB toolbox designed for the detection of spatiotemporal patterns in neural population activity. Based on 'optical flow analysis', the toolbox reveals the emergence of characteristic patterns in the activity of the networks, and their dependence on DIVs and surface. Results indicate that development and surface properties change the connectivity of the network, which gives rise to macroscopic signatures such as characteristic patterns of activity.
Keywords: biophysics, neuronal networks, spontaneous activity, optical flow, pattern detection.
SDGs: This work is related to the sustainable-development goals (SDGs) 3, 4 and 9.

I. INTRODUCTION

Living neuronal networks, from small systems such as neurons grow *in vitro* to model animals such as the C. Elegans or the Zebrafish, are investigated in physics of complex systems to understand the functioning of the brain, particularly in universal aspects such as spontaneous activity, i.e., the ability of neurons to activate without any external stimulation, or the spatiotemporal propagation of information, i.e., the transmission of activity from neuron to neuron within the neuronal network.

To investigate these aspects in detail, neuronal cultures have emerged as an accessible, and highly interesting, laboratory toolbox [1]. They consist of neurons plated on a surface that contains the appropriate nutrients for the cells to grow and develop into a neuronal network. The surface used as a substrate for the neurons can be flat (such as glass), filled with electrodes, or topographically patterned, meaning it has small depressions which affect neuronal connections and consequently the overall connectivity details of the network [2].

Neuronal cultures exhibit spontaneous activity, which is the capacity of the neurons in the network to activate by themselves thanks to intrinsic noise and connectivity [1]. This activity propagates throughout the network, but the propagation details, e.g., a circular wave or a spiral, may depend on the connectivity of the network or the length of the connections. To monitor this spontaneous activity in cultures, the scientific community mainly uses two methods: fluorescence calcium imaging [3] and multielectrode arrays (MEAs) [4].

Calcium imaging is based on the optical detection of neuronal activations as increases of fluorescence in neurons, caused by the increase in intracellular calcium concentration when neurons fire an action potential. The neurons are previously loaded with a calcium indicator, a compound that exhibits fluorescent properties when bound to calcium and that allows to detect activity.

MEAs, on the other hand, measure the electrical signals of neurons when they fire an action potential, thanks to an array of electrodes embedded in the substrate where neurons grow. Calcium imaging offers high spatial resolution, but has poorer time resolution since it is limited by the kinetics of the indicator. MEAs offer great time resolution but have bad spatial resolution since neurons cannot be seen due to electrodes' opacity.

In either case, calcium imaging or MEAs, however, spatiotemporal activity fronts can be tracked and analyzed. The goal of the present work is to classify such patterns in experimental data from Dr. Soriano laboratory, and using the framework of 'optical flow analysis' published by Townsend and Gong [6].

II. EXPERIMENTAL BACKGROUND

Neuronal cultures in the present study came from either rat primary neurons or human induced pluripotent stem cells (hiPSCs) [5]. The types of substrates used were a flat surface, electrodes, or a topographical substrate containing depressions in the form of randomly positioned squares with varying sizes, which we named 'squares' [2]. For the data obtained with calcium imaging, we used rat neurons placed in a flat surface and the 'squares' patterns, and recorded the activity of 2 batches of cultures for each surface type at different days *in vitro* (DIV). For the data obtained with MEAs, we used rat neurons and hiPSCs cells placed on a flat MEAs surface and recorded one culture of each type for multiple DIVs. The used data is summarized in Table I.

The overall motivation to analyze the described experimental data is: (i) to see whether there are distinct spatiotemporal patterns that emerge in the spontaneous activity of the neuronal networks, and (ii) to investigate the effect that different types of neurons and substrates, as well as the development of the cultures along DIVs,

have on these activity patterns.

MEA	Flat	
Human	DIV 37, 65, 98	
Rat	DIV 10, 17	
Calcium	Flat	Squares
Rat	DIV 7, 10, 12	DIV 7, 10, 12
	DIV 7, 10, 12, 14, 17	DIV 7, 10, 12, 14, 17

TABLE I: Summary of experimental data used.

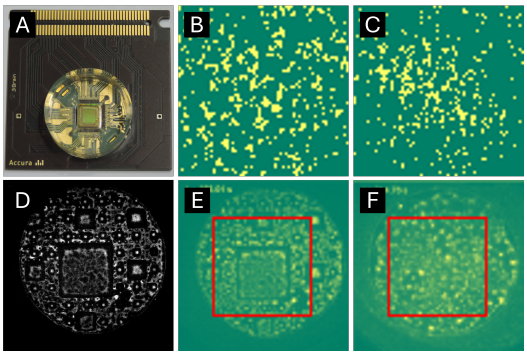

FIG. 1: Overview of the data used and the detection methods. (A) Picture of the MEA chip. The neurons are placed on the square inside the circle, which contains the electrodes. (B) Frame of an activity matrix for human neurons placed on a flat MEA surface. (C) Same as (B) but for rat neurons. (D) Frame of a calcium imaging video for rat neurons placed on a patterned surface. (E) Frame of an activity matrix for rat neurons placed on a patterned surface. (F) Same as (E) but for a flat surface.

III. METHODS

A. Introducing 'optical flow analysis'

Optical flow analysis consists of determining the motion between consecutive frames of data by tracking changes on the images. This is done by solving two constraints. The first constraint states that the same data D is present at time t and $t+\delta t$, but it may shift in space depending on the x and y components of the velocity field, u and v respectively, of the data, i.e.,

$$D(x+u\delta t, y+v\delta t, t+\delta t) - D(x,y,t) = 0. \quad (1)$$

In a first order approximation, this can be expressed as:

$$E_d = \frac{\partial D}{\partial x}u + \frac{\partial D}{\partial y}v + \frac{\partial D}{\partial t} \approx 0, \quad (2)$$

where E_d is the error in the conservation of data. The second constraint states that the velocity fields are smooth and continuous, so that

$$E_s^2 = |\nabla u|^2 + |\nabla v|^2, \quad (3)$$

where E_s is the error in the smoothness of the velocity. The velocity field $w(x,y,t) = (u(x,y,t), v(x,y,t))$ can be then uniquely defined by minimizing both errors, i.e.,

$$\min_{u,v}\{\int\int[\rho(E_d)^2 + \alpha\rho(E_s)^2]dxdy, \quad (4)$$

where α is a smoothing parameter and $\rho = \sqrt{x^2+\beta^2}$ is a penalty controlled through a parameter β. Eq. (4) is solved through its Euler-Lagrange equations.

For the purpose of analyzing the activity patterns of neuronal networks, we used the `NeuroPattToolbox` MATLAB toolbox [6] which detects spatiotemporal patterns in neuronal networks. This toolbox uses a 3D matrix $I(x,y,t)$ as input data, where I is the intensity of the signal, (x,y) the position in Cartesian coordinates of each dynamic element (neurons) and t the corresponding time of activation. Being I a matrix, x, y and t are discrete and evenly spaced. The time dimension t is associated to frames in a recording of neuronal activity.

Our data, regardless of the type of neuron (primary or hiPSC), culturing surface or recording method used, had a similar structure. It consisted of noise with very little activity and sudden bursts of strong neuronal activity (Fig. 1). The frequency, intensity and duration of the bursts changed, and we speculate that their spatiotemporal structure also changed, e.g., from slow circular wave to fast synchronous dynamics. In order to analyze these spatiotemporal patterns, we followed 3 steps: (i) data preprocessing to convert the recordings into a matrix, (ii) selection of bursts of high activity in the network, and (iii) analysis through the aforementioned toolbox.

B. Preprocessing of MEA data

MEA recordings consist of a list of the electrode number and the time of activation of every event detected. To transform this data into a matrix we used the grid of the MEA to convert the electrode number into x and y positions, starting with electrode number 1 at the top left corner with coordinates $(1,1)$ and ending at electrode 4096 at the bottom right corner with coordinates $(64,64)$. To discretize time, we chose a certain time interval, and all activations that happened within that time belonged to a single frame. As the MEA does not offer a value for the intensity of the activation we used 1 for the presence of activity and 0 otherwise.

The duration of a frame was chosen case by case to achieve the optimal time resolution. Using the periods of high activity in the network we tested different frame durations to see which ones allowed sufficient temporal resolution but also accumulated enough activations, since a higher amount of activations in a frame shortened the time needed to estimate the optical flow.

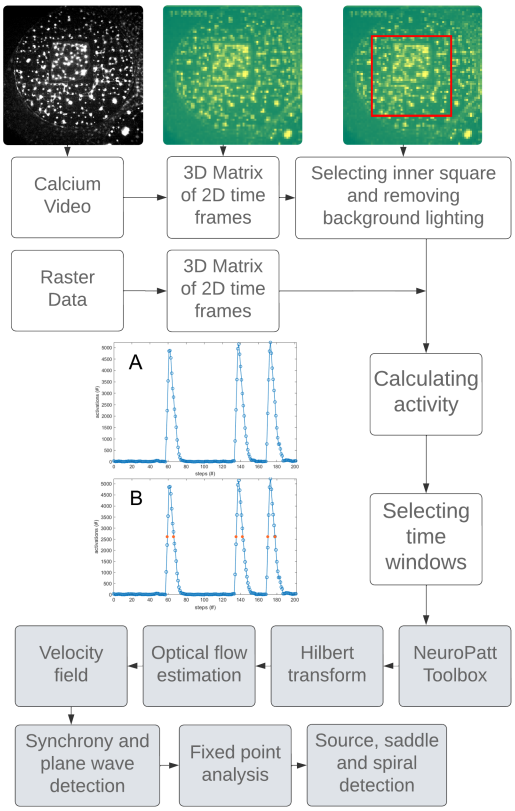

FIG. 2: Flowchart of data analysis. Main figure: data preprocessing and input. Inset panels (A)-(B): Examples of network activity evolution (blue) and detected events of strong network activity (red dots).

Proper mathematical definitions were given for *electrode activation* and *activity episode*. An activation was defined, for the MEA data, as an event recorded by a single electrode. A set of activations A in a time window Δt defined an 'activity episode' by quantifying the number of electrode activations within this time.

The quantity A was then evaluated along a moving widow to detect episodes of strong activity in a smooth manner. The size of the window and its moving step was set for each particular dataset. The ultimate goal was to detect events in which activity appeared, became strong and then stopped, which actually indicated a macroscopic propagating wave or a *burst* of activity.

Next, we isolated the spikes within these bursts of high activity. For that, as illustrated in Fig. 2A-B, we selected all the time steps at which activity was above half of the maximum activity and chose the time steps at the beginning and end of the half peak. Choosing only the values of activity above half of the maximum ensured that there were enough activations in every frame for the toolbox to analyze the data in an adequate amount of time.

The chosen time steps were then converted onto frames by taking the frame that contained the first time step and the frame that contained the last time step plus the window. The resulting frames contained the beginning and end of the high activity episodes. Then, all these strong activity episodes were given as input data to the `NeuroPattToolbox` toolbox to extract the spatiotemporal patterns.

C. Preprocessing of calcium imaging data

The data obtained from calcium imaging is a video in which neuronal firings appear as bright spots on the images. To transform the video into a matrix, we first converted the video to grayscale, divided each image into a 70×70 grid of regions of interest (ROIs), and averaged the brightness of all pixels inside each ROI, repeating for every frame. We focused only on the square inscribed inside the circular culture (Fig. 1E-F), since the toolbox assumes the input matrix to be rectangular.

In the experiments there were light artifacts in the form of ambient illumination (from the laboratory) and background fluorescence (from the neurons at rest), which had to be corrected. We took the intensity over time of a single ROI and performed a parabolic regression on it. The regression was made after removing the peaks in intensity so it would follow only the base brightness that was independent of the network's activity. After subtracting the regression to the original intensity, we obtained a brightness proportional to neuronal activity.

To isolate the bursts of high activity we defined again *activation* and *activity episode* for the calcium data, this time using the intensity of fluorescence to consider neuronal activations and strong peaks of global activity to consider bursting episodes. Then, everything was the same as in the MEA data.

D. Neuropatt Toolbox

This toolbox performs an analysis of the spatiotemporal patterns that appear in neuronal networks. For our analysis, we focused only on the type and number of patterns that appeared in each investigated culture.

NeuroPatt is able to detect 7 different types of patterns, as described in Fig. 3. The first step in the detection algorithm is to perform an optical flow estimation that produces a velocity field, which is then analyzed. First as a whole to determine the appearance of plane waves and synchronies, and then through *fixed point* analysis, i.e., regions in space where the velocity field is zero, such as sinks, sources, spiral-ins and outs, and saddles.

Given a velocity field $w(x, y, t) = (u(x, y, t), v(x, y, t))$ with u the x component of the velocity and v the y component, a plane wave is detected by introducing parameter

$$\phi(t) = \frac{||\sum_{x,y} w(x, y, t)||}{\sum_{x,y} ||w(x, y, t)||}, \tag{5}$$

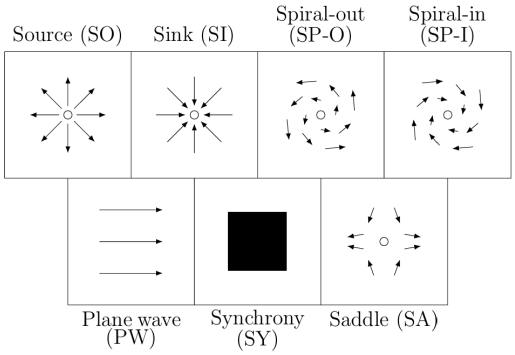

FIG. 3: The different patterns in the NeuroPatt toolbox.

which approaches 1 when all velocity vectors point in the same direction. For $\phi(t)$ above a threshold $\phi_T = 0.7$, then a plane wave is considered dominant and accepted.

For the detection of synchronies, the phase θ of the intensity I is obtained through the Hilbert transform $I + i\hat{I} = Ae^{i\theta}$, where $\hat{I}$ is the transform of the intensity. Then we define another parameter

$$R(t) = \frac{1}{N}\left|\sum_{x,y} e^{i\theta(x,y,t)}\right|, \tag{6}$$

where N is the number of ROIs. $R(t)$ approaches 1 as the phase of every space takes the same value, and when the value of $R(t)$ surpasses a threshold $R_T = 0.7$, then synchrony is considered strong and accepted.

Fixed point analysis is performed by finding the Jacobian matrix of a fixed point as

$$\begin{pmatrix} \frac{\partial u}{\partial x} & \frac{\partial u}{\partial y} \\ \frac{\partial v}{\partial x} & \frac{\partial v}{\partial y} \end{pmatrix}. \tag{7}$$

Depending on the trace (τ) and determinant (Δ) of the matrix, we can characterize the rest of the patterns, which are: *sink* ($\Delta > 0$, $\tau^2 > 4\Delta$, $\tau > 0$); *source* ($\Delta > 0$, $\tau^2 > 4\Delta$, $\tau < 0$); *spiral-in* ($\Delta > 0$, $\tau^2 < 4\Delta$, $\tau > 0$); *spiral-out* ($\Delta > 0$, $\tau^2 < 4\Delta$, $\tau < 0$); and *saddle* ($\Delta < 0$).

To help validating the results, NeuroPatt provides a generator of synthetic data to compare the results obtained from real recordings to artificial noisy data with similar statistical properties. For that, for every ROI, a time series of white noise with the same mean and standard deviation of the experimental data is created. Then, synthetic data is analyzed in the same way as the experimental one, and the obtained results are compared.

IV. RESULTS AND DISCUSSION

A. Experimental and synthetic data

To check whether the results we obtained depended on the activity of the network or if they were created by noise, we compared the detected patterns in experimental and synthetic data. We can see in Fig. 4 that, in general, all patterns had stronger rates of presence in the experimental data than in the synthetic one, which suggests that the detected patterns were not just noise or analysis artifacts. Saddles (SA) and spirals (SP) were conflicting, and often their presence appeared to be more relevant in the synthetic data, but spirals were ultimately used in our analysis since we considered they were relevant. In Fig. 4 we only provided 2 examples of analyzed data, but this trend persisted in all cultures and DIV.

As Fig. 4 shows, MEA (left) and calcium data (right) could be properly analyzed, and both detected different patterns. We found that the cultures grown on MEA and flat surfaces (left) had stronger differences between experimental and synthetic data than those placed on the 'Squares' surface and recorded with calcium (right). Additionally, as we will show later, there were interesting differences along DIV.

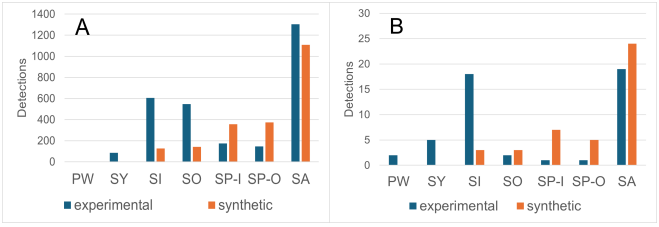

FIG. 4: Comparison of the presence of patterns between experimental (blue) and synthetic (orange) data. (A) hiPSCs on a flat MEA surface. (B) Rat neurons on a patterned surface recorded through calcium imaging.

B. Evolution of the number of patterns

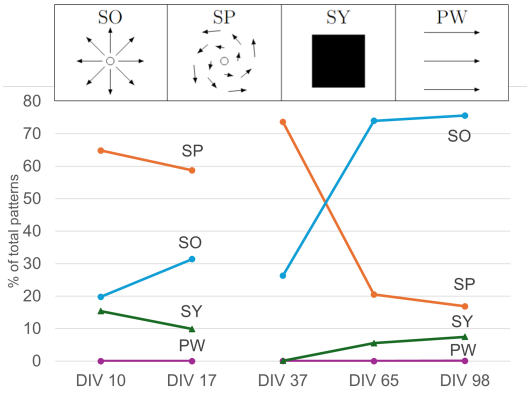

FIG. 5: Percentage of pattern type along DIVs for the MEA data. Left: rat neurons. Right: hiPSCs neurons.

To analyze the evolution of the activity of the networks we focused on the number of patterns of each type. For clarity, we put together sinks and sources into the 'source' category, and spiral-ins and outs into the 'spiral' category. The reason is that these patterns were similar in form and had similar appearance rates. Then we calculated the percentage of presence of each pattern.

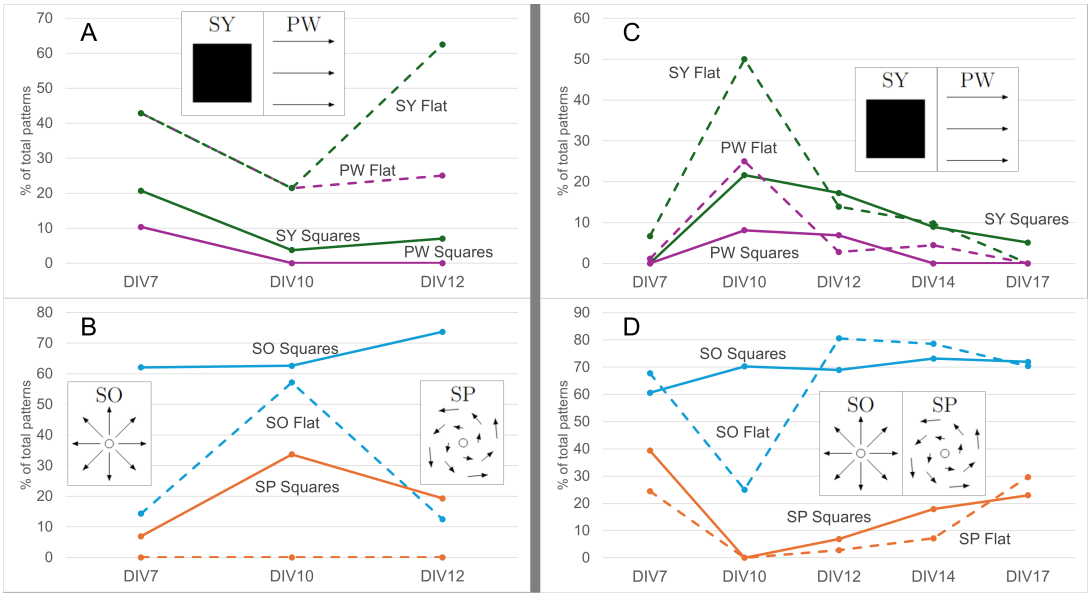

FIG. 6: Percentage of pattern type along DIVs for the first (left) and second (right) batches of cultures of calcium data. (A) % of PW and SY of the first batch. (B) % of SO and SP of the first batch. (C)-(D) Same as (A)-(B) but for the second batch. The dotted line indicates a flat surface and the continuous line a patterned surface.

Fig. 5 compares the dominant patterns between rat (left) and human (right) cultures in a MEA system, showing that characteristic patterns appear in their spontaneous activity, and that percentage of each pattern changed was somehow maintained across cell types, but that clearly changed along DIV, with 'source' (SO) becoming stronger as compared to 'spiral' (SP). This suggests that the biological network changes itself over time, which brings about changes in connectivity that have a macroscopic evidence through the activity patterns.

Regarding calcium data on rat cultures, Fig. 6(A)-(B) shows a first batch comparing substrate type, with the dominant patterns separated in two panels for clarity. Interestingly, the data shows that the substrate had an effect, with flat surfaces being dominated by 'synchronous' (SY) and 'planar waves' (PW), while patterned substrates being dominated by 'spiral' and 'source' (SO) patterns. Thus, substrate patterning affects connectivity and the overall dynamics of the network. Interestingly, a second batch of calcium data, shown in Fig. 6(C)-(D), reveals that results are consistent across realizations, but that one has to consider, for a strong assessment of dominant patterns, several batches and average over the data.

V. CONCLUSIONS

The present work has demonstrated that optical flow analysis can be successfully applied to the analysis of spontaneous activity of neuronal networks, being able to detect a variety of spatiotemporal patterns in data as different as MEAs and calcium imaging. More importantly, the results show that the detected patterns change when the substrate in which neurons grow is altered, or when the connectivity of the network evolves due to development. The latter case is very important, since it indicates a reorganization of the connectivity of the network that translates into macroscopic observables, an aspect that is in general very difficult to explore.

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Classificació dels patrons d'activitat en xarxes neuronals vives mitjançant flux òptic

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Resum: Els cultius neuronals són una manera accessible d'observar i modelitzar el comportament de xarxes neuronals vives. En aquest estudi, l'activitat espontània observada en aquests cultius és analitzada amb la finalitat de trobar patrons espaciotemporals en l'activitat espontània, com ara sincronies o fronts espirals. Neurones humanes i de rata, cultivades en superfícies planes o alterades, han sigut analitzades al llarg de diferents dies *in vitro* (DIV) utilitzant Neuropatt, una eina creada amb MATLAB per la detecció de patrons espaciotemporals en l'activitat neuronal. Aquesta eina, basada en el 'flux òptic', revela l'aparició de patrons en l'activitat de les xarxes i la seva dependència en els DIV i superfície. Els resultats indiquen que el desenvolupament de la xarxa i les propietats de la superfície canvien la connectivitat de la xarxa, causant efectes macroscòpics com ara els patrons en l'activitat.
Paraules clau: biofísica, xarxes neuronals, activitat espontània, flux òptic, detecció de patrons.
ODSs: Aquest TFG està relacionat amb els Objectius de Desenvolupament Sostenible (ODS) 3, 4 i 9

Objectius de Desenvolupament Sostenible (ODSs o SDGs)

1. Fi de la es desigualtats		10. Reducció de les desigualtats	
2. Fam zero		11. Ciutats i comunitats sostenibles	
3. Salut i benestar	X	12. Consum i producció responsables	
4. Educació de qualitat	X	13. Acció climàtica	
5. Igualtat de gènere		14. Vida submarina	
6. Aigua neta i sanejament		15. Vida terrestre	
7. Energia neta i sostenible		16. Pau, justícia i institucions sòlides	
8. Treball digne i creixement econòmic		17. Aliança pels objectius	
9. Indústria, innovació, infraestructures	X		

El contingut d'aquest TFG es relaciona amb l'ODS 3, ja que l'estudi del comportament de les neurones té beneficis per la salut com ara ajudar a combatre malalties neurodegeneratives, entre d'altres. També es relaciona amb l'ODS 4, i en particular amb la fita 4.4, ja que contribueix a l'educació a nivell universitari. Finalment, sent un treball d'investigació, es relaciona amb la fita 9, concreatament la fita 9.5, que busca augmentar la investigació científica.