The role of major signaling pathways in vertebrate neurogenesis

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The study of developmental processes often relies on image analysis, given the key influence of spatial cues such as morphogenes or contact inhibition in the regulation of stem cell differentiation. Unfortunately, imaging these dense three-dimensional developing organs results in reduced resolution, and reliable quantification of these images becomes very challenging.

To overcome these limitations, we have developed a novel Object Segmentation, Counter and Analysis Resource (OSCAR) specifically designed to quantify three-dimensional images from densely packed biological samples, such as developing organs in vertebrates.

In this contribution, we will explain the basics of how how our tools is able to outperform other commercial solutions, and apply OSCAR to study the zebrafish developing retina, providing a quantitative characterization of the dynamics of the vertebrate retinogenesis in space and time with unprecedented accuracy.

Combined with small molecule inhibition treatment and a branching process mathematical formalism, we are able to unveil the role of major signaling pathways, such as HH, Wnt and Notch, in the regulation of the balance between proliferation and differentiation of neural stem cells into terminally differentiated neurons.

Our framework is not only valid for retinas of smaller vertebrates, and it is also very capable of extracting the number and location of all cells in other types of 3D cultures, such as neurospheres from mouse cerebral cortex cultured *in vitro* (see Fig. 1 for an example) or even retinal organoids from human iPS cells.

In the context of the developing retina, our results show that the Sonic Hedgehog pathway, a master regulator in the formation of potentially all organs in vertebrates, induces differentiation and cell cycle progression, while the Wnt-Frizzle pathway (Wnt) promotes cell cycle progression without affecting the cell cycle. Another major regulator of developmental processes, the Notch-Delta pathway, strongly reduces differentiation when inhibited, but it does so via arresting cell in the cell cycle. Overall, the combination of out image analysis and our branching process mathematical model allows us to obtain a clear picture of the role of each major signaling pathway in vertebrate neurogenesis.

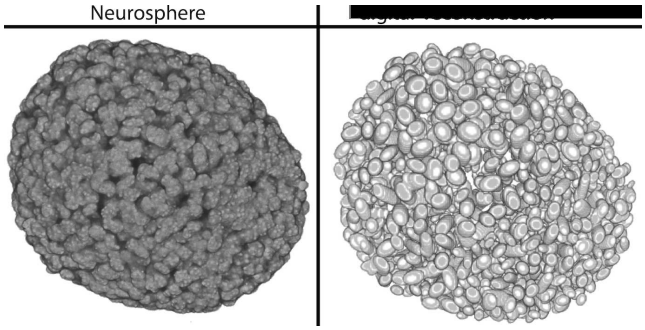

Fig. 1. Example of the capabilities of our framework OSCAR in extracting the location of each cell in a crowded three-dimensional tissue such as a neurosphere. Left) Original 3D image. Right) Digital representation using OSCAR output.

[1] Mario Ledesma-Terrón, Diego Pérez-Dones and David G. Míguez , *In preparation* (2023).

[2] https://www.cbm.uam.es