Fluorescence Correlation Spectroscopy as a tool in Developmental Biology

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Fluorescence Correlation Spectroscopy (FCS) is a widely used tool for the characterization of molecular dynamics. It relies on the recording of fluctuations in fluorescence from a small observation volume. These fluctuations carry information about the processes that cause them. In its most common form, one observes the diffusion of fluorescent particles in and out of femtoliter-sized confocal volume. The resulting fluctuations carry then the information about the average number of particles in the volume as well as their diffusion coefficient. Over the years many different FCS modalities, using different illumination profiles, recording multiple wavelengths, reducing noise and background of the recordings, have been developed. This provides the user nowadays with a plethora of modalities from which to choose for a particular problem and which can be combined to maximize the information that can be obtained from a particular sample.

Here we will discuss the use of a combination of three different FCS modalities – confocal FCS, confocal Fluorescence Cross-Correlation Spectroscopy (FCCS) and single plane illumination microscopy FCS (SPIM-FCS) – to investigate the dynamics and interactions of Wnt3 in live zebrafish. Wnt3 is a morphogen expressed in the developing brain and the dorsal spinal cord and plays an important role in zebrafish neurodevelopment. The subcellular distribution of this protein and its interaction with receptors are not fully understood. For its study, we used zebrafish transgenics expressing Wnt3-EGFP under a 4kb *wnt3* promoter.

We first investigated by confocal microscopy where Wnt3 is produced and where cells-targets are. We then determined by SPIM-FCS the presence of Wnt3 on cell membranes where it is localized to particular lipid domains. This is important for secretion but also for signaling as its putative Frizzled receptors (Fzd) have been found in such domains [1,2]. Using FCS, we then asked how Wnt3 is reaching its target cells by determining whether Wnt3 is secreted and can be found in the interstitial spaces, between cells, where it can then diffuse to the target cells, or whether it is membrane bound and requires other transport mechanisms (e.g. cytonemes) [3, 4]. And finally, we used FCCS to determine the dissociation constant (Kd) for the Wnt3/Fzd1 pair. Taken together these FCS modalities represent a quantitative approach in analysis of morphogens function in vertebrates *in vivo*.

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