# Single-molecule microscopy in zebrafish embryos

Góra, R.J.

# PROPOSITIONS

accompanying the thesis

## Single-Molecule Microscopy in Zebrafish Embryos

1. The H-Ras protein occurs, in epidermal cells of zebrafish embryos, in at least three different states: a fast-diffusing, a slow-diffusing, and an immobile state, and the size of the subpopulations occurring in these states, their diffusion rate and confinement zone depend on the activation status of the protein and the architecture of the cells (**Chapters 2 and 3**).
2. Slow-diffusing H-Ras molecules do not exist in only one dynamic state, but alternate between multiple mobility states of varying duration, diffusion, and confinement (**Chapter 3**).
3. Differences between cells within the same tissue of the zebrafish embryo contribute largely to the overall variability of mobility patterns of H-Ras and the glucocorticoid receptor, often even more than differences between individual embryos (**Chapters 2 and 4**).
4. An inverted selective plane illumination microscopy configuration can be used for detecting single fluorescent proteins (e.g., the glucocorticoid receptor) in zebrafish embryos, and enables a sample mounting approach that is superior in terms of sample viability and mounting flexibility compared to other light-sheet fluorescence microscopy setups (**Chapter 4**).
5. In single-molecule microscopy studies exploring the impact of factors such as stress, the metabolic status or developmental conditions on protein dynamics in zebrafish embryos, mounting procedures and sustaining the embryo's viability will be the biggest challenges.
6. The selection of a microscopy technique for applications in a living organism, e.g., a zebrafish embryo, is always a trade-off between five aspects: imaging speed, photobleaching and phototoxicity, spatial resolution, signal-to-noise ratio, and the physical coverage of the biological specimen (Keller P.J. *Methods,* 2013; Abu-Siniyeh A., Al-Zyoud W. *Lab Anim Res,* 2020).
7. When the dynamic behaviour of molecules is studied using single-molecule microscopy, the temporal resolution of the used microscopy setup will determine which subpopulations of molecules can be detected.
8. In research aimed at reducing phototoxicity and photobleaching rates of fluorescent molecules, the emphasis has been put on designing new fluorophores, but the development of novel imaging techniques may be a more successful approach (Tosheva K.L., et al. *J Phys D Appl Phys*, 2020; Icha J., et al. *Bioessays*, 2017, Donnert G., et al. *Nat Methods*, 2007).
9. Good mentoring skills of supervisors encourage students to embark on a career path in academic research.
10. A proper balance between collaboration and competition between researchers is a driving force of scientific progress.

**Radosław Jakub Góra, Leiden, 23 November 2022**