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Enhancing in vivo-imaging in medaka by objectively improving pigmentation, anesthesia and fluorescent proteins

Medaka (Oryzias latipes) is amenable to *in vivo*-imaging by light-sheet microscopy due to its comparably slow development, its very large available toolbox and mostly transparent embryos. Its lifelong growth allows extensive studies of stem cells. However, following these stem cells and their descendants by *in vivo*-imaging is very challenging and imaging conditions needed optimization.
In order to improve these imaging conditions we addressed the largest challenges for *in vivo*-imaging in medaka: retinal and peritoneal pigmentation, anesthesia and the choice of fluorescent protein. We addressed the latter two by a medium-throughput screen using 96-well plates and time-lapse imaging in an Acquifer plate imaging machine.
The retinal and peritoneal pigmentation was largely reduced by introducing a double knockout of *Oca2* and *Pnp4a* using the CRISPR/Cas9-system. This double knockout abolishes retinal pigment epithelium and iridophore pigments completely.
Anesthesia was improved by substituting the commonly used MS222 with injections of α-Bungarotoxin mRNA, as suggested by similar experiments in zebrafish.
Fluorescent proteins were scored in green and red. The results indicate that eGFP and mCherry are the best conservative choices, if you are considering *in vivo*-fluorescence and the available toolsets such as antibodies or nanobodies. However, mVenusNB and mGFPmut2 showed a higher fluorescence intensity *in vivo* compared to eGFP and can be used depending on the used analysis methods.
During this study we improved imaging conditions in medaka dramatically and are thus able to perform light-sheet microscopy of the developing retina, brain, gills and inner organs, without the use of toxic chemicals. This is achieved by using a combination of pigment double knockout fish, α-Bungarotoxin mRNA anesthesia and suitable fluorescent proteins.

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