New insights into one/two-photon properties of mScarlet fluorescent protein, its versatile use in living and fixed cells and tissues and in organoids

*Tripal P., Fraass T., Schmid B., Palmisano R.**

Optical Imaging Centre Erlangen, Friedrich Alexander University Erlangen-Nürnberg (FAU), Germany

* Corresponding Author: ralf.palmisano@fau.de

With the advent of mScarlet[1], a novel bright monomeric red fluorescent protein (FP), the tool-box for cellular imaging has been extended with a promising red fluorescent variant to replace limited red FPs such as RFP or mCherry. We have investigated its localisation accuracy on different intra-cellular structures such as cytoplasm, actin, α-tubulin, peroxisomes, H2A or mitochondria, compared to established sub-cellular stainings like SiR-DNA, Phalloidin, Mitotracker, Cellmask, anti-α-tubulin antibody, BacMam. Furthermore, we investigated mScarlet's performance using different fixation methods (PFA, Methanol e.g.). Our observation showed no evidence of any unexpected localisation or unwanted artifacts on fixed cell fluorescent imaging.
We also investigated its multiphoton properties and could show that the 3 mScarlet variants are susceptible to two-photon excitation and again compared its localisation and physical properties to LifeAct RFP. Thus we also examined mScarlet as a STED compatible FP. Although it bleaches after repetitive imaging it could also prove useful for 2D-STED in combination with the Rescue-STED, where only one acquisition frame is needed. However, we still have to investigate the mScarlet H and I variant, too.
Meanwhile we also established stable HeLa cell lines of pLifeAct mScarlet-H, pMTS mScarlet-H, pmScarlet-I peroxisome and pmScarlet-H H2A, with pmScarlet-H α-tubulin, pmScarlet NES, pLifeAct mScalet-I and mScarlet in final rounds of selection. Finally, we are in the process to investigate the use of transiently mScarlet infected organoid structures to make use of mScarlet's superior properties in long-term live cell and organoid observation.
From our findings we conclude mScarlet to have the power to be the new standard red-fluorescent protein in particular for single or multi-photon live cell imaging and furthermore also to become extremely helpful for future in-vivo imaging on small animals like mouse or light-sheet based imaging technologies. We are currently in the process of generating more insight into these two technologies.

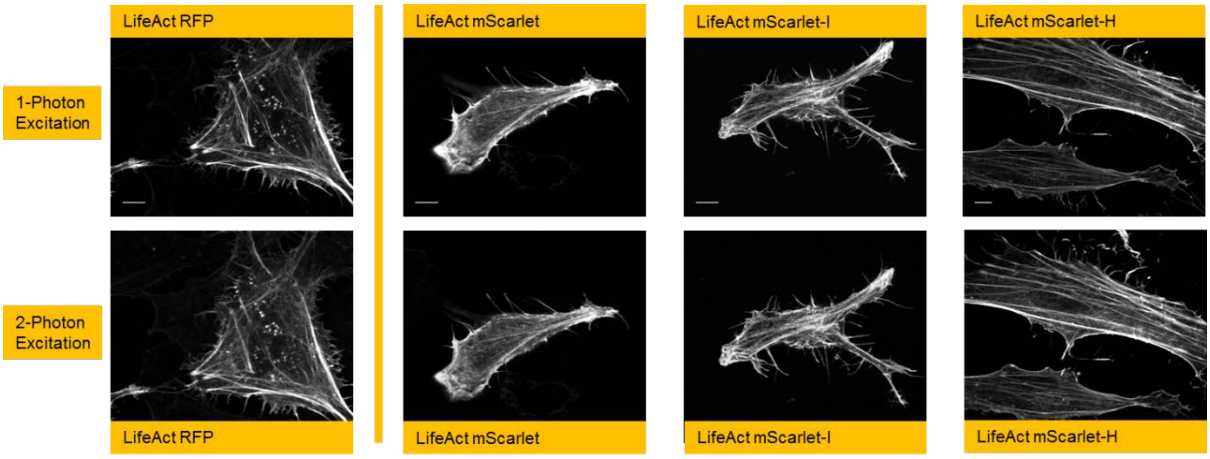

Figure1: The three mScarlet variants fused to LifeAct actin binding protein and compared with commercially available LifeAct RFP

1) Bindels DS, Haarbosch L, van Weeren L, Postma M, Wiese KE, Mastop M, Aumonier S, Gotthard G, Royant A, Hink MA, Gadella TW Jr. *mScarlet: a bright monomeric red fluorescent protein for cellular imaging*
Nat Methods. 2017 Jan;14(1):53-56. doi: 10.1038/nmeth.4074.