

Fig 1: Multi-label spectral unmixing of multiple fluorophores in a single mouse.

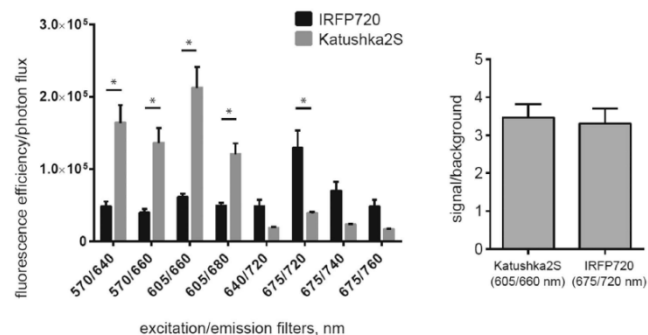


Fig 2: Katushka2S and iRFP720 SNRs.

Genetically encoded far-red and near-infrared fluorescent proteins (RFPs) enable efficient non-invasive *in vivo* imaging in studies of tumorigenesis, embryogenesis, and inflammation in model animals. In this article, a thorough evaluation and comparison of currently available RFPs is outlined and it was demonstrated that:

- The signals of various RFPs can be spectrally unmixed based on different signal-to-noise ratios (SNR) in different channels (Fig 1).
- When HEK293T cells were transiently co-transfected with Kasushka2S, eqFP650, mCardinal, mNeptune 2.5 as indicated and normalized to firefly luciferase photon flux for each implant, **Katushka2S** (Evrogen.com) produced the brightest and fastest maturing fluorescence in all experimental setups (Fig 3).
- At the same time, SNRs for Katushka2S and near-infrared bacterial phytochrome, **iRFP720** (Vladislav Verkhusha, Addgene.org) were comparable in their optimal channels (Fig 2).

The authors conclude that due to their distinct spectral and genetic characteristics the pairing of Katushka2S and iRFP720 are an optimal far-red/near-infrared fluorescent protein combination to inform on molecular signals and cell populations in dual color, whole body imaging studies.

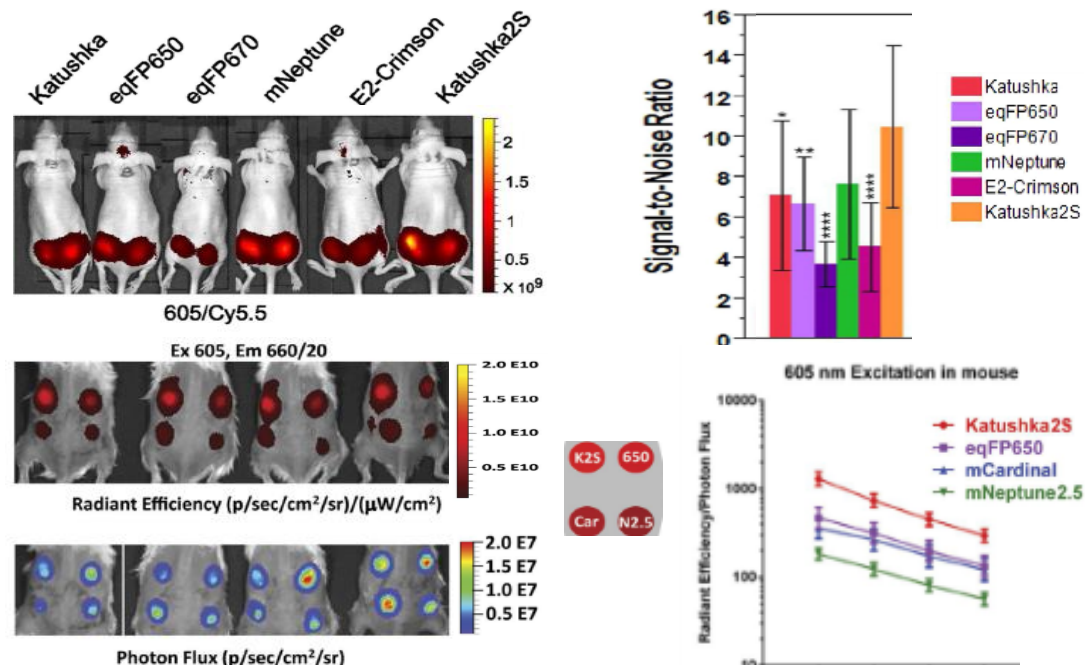


Fig 3: Comparison of RFPs in whole-mouse imaging.