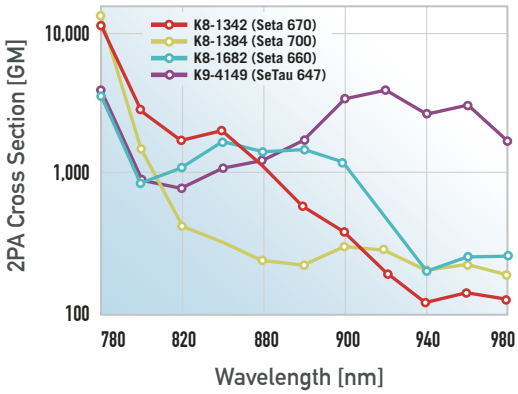
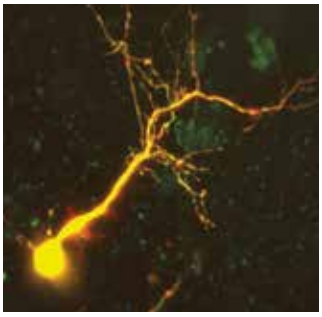
Superior Probes and Labels for Two-Photon Microscopy

SETA BioMedicals has developed several Two-Photon-(2P)-probes and labels based on squaraines and squaraine rotaxanes with extremely high Two-Photon-Absorption (2PA) cross sections and emission between 500 and 700 nm for *in vivo* imaging applications.



2-photon action cross sections for several squaraines and squaraine rotaxanes



2-photon image of neuron loaded with SeTau-647

While the 2PA cross sections of common fluorescent labels are in the order of 100 GM (2PA cross section of the di-anioic form of fluorescein in water at pH 13 is 36 GM and for Rhodamine B 150 GM for excitation at 800 nm) and even some of the best 2P labels published have 2PA cross sections of only several hundred GM, Seta and SeTau dyes have 2PA cross sections in the order of thousand to several thousand GM.

The 2PA wavelengths and cross-sections for selected Seta and SeTau dyes are provided in the table below.

Product Number	Product Name	Maximum 2PA Wavelength [nm]	λ em [nm]	2P-Excitation Cross-Section [GM]
K7-547	SeTau-405-NHS	750-850	518	>100
K8-1672	Seta-646-NHS	840	660	480
K9-4149	SeTau-647-NHS	920	695	3700
K8-1682	Seta-660-NHS	900	675	1625
K8-1342	Seta-670-NHS	840	690	1925
K9-4150	SeTau-647	920	693	>3000
K9-4145	SeTau-633	900-920	683	>1000

Seta and SeTau dyes offer ideal fluorescence properties for *in vivo* two-photon imaging. SeTau dyes have 2P cross sections and photostability approaching those of quantum dots, but with molecular weights similar to other organic dyes, allowing labelling of subcellular structures at low concentrations and with reduced functional impact. Importantly they are non-toxic and are well-suited for long-term neuronal imaging. The demonstrated brightness and stability of these dyes promise to extend the limits of fluorophore concentration, imaging rate, illumination depth, and imaging duration for *in vivo* two-photon microscopy [1].

[1] Podgorski K. et al. Ultra-bright and -stable red and NIR squaraine fluorphores for in-vivo 2-photon imaging. PLOS ONE 7(12): e51980 (2012).

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