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An unanticipated result is that correction of phase beyond third order dispersion has a measurable impact on the excitation efficiency for pulses as long as 50 fs in duration. This is of practical importance as most commercial Ti:Al<sub>2</sub>O<sub>3</sub> lasers sold for biological imaging purposes have durations in the range of 50-100 fs. Moreover, the improvement in the two photon fluorescence signal scales approximately as the inverse of pulse duration for Rhodamine B while the fluorescence measured from the TagRFP sample increases more dramatically with higher-order correction (Fig. 4).

A pragmatic point concerns the throughput of the pulse shaping device. The intensity of the fluorescence signal varies as the square of the average excitation power [21], yet is linearly proportional to the second order coherence. As an example, if one uses a pulse shaper with 50% throughput, the fluorescence intensity will fall to 25% of the original level, assuming the same pulse duration. Thus it is important to ensure that the gain in fluorescence from the reduction of the pulse duration will offset the loss in fluorescence due to throughput losses in the pulse shaping apparatus.

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