Photometry - technical overview

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## Technical overview

This overview is focused on the technicalities of bulk photometry recordings.

### Bleaching, decay and temporal drifts

Bulk fluorescence signals are unstable by nature. Even if the bleaching of GcAMP is limited compared with 2-photon imaging that uses much strong light beams, it still contributes to signal decay. Other sources of signal decay are less well understood, but they may involve all or part of the following mechanisms: 1. **Reduction in apparatus autofluorescence**. Typically, the patchcords used for photometric recordings are autofluorescent (even the “low autofluorescence” versions tailored to photometry). This type of autofluorescence is mostly driven by the connection between different elements of the recording apparatuses, and it tends to decrease over time. Recordings without any connection to a living animal can highlight this phenomenon. 2. **Reduction in GcAMP-independent tissue autofluorescence**. Brain tissue is intrinsically autofluorescent and this autofluorescence is also susceptible to bleaching. 3. **Green-to-red photoconversion of GCaMP**. This phenomenon is less established but it has been reported for various versions of GCaMP 4. **Reduction in light power over time**. This issue is less common but it can arise when LED cells are “burnt” over the course of an experiment. With old lasers, the temporal drift can be unpredictable (which is to be avoided at all cost!)

Signal decay can unfortunately happen at different rates for different excitation frequencies (e.g. 405, 470, 565, etc.), which generally requires to correct temporal drifts on a *per* channel basis before attempting movement artifact correction.

### Isosbestic signal and other correction methods

### Filtering

### Origins of the signal