Bioluminescence Microscopy: A tool to record the kinetics of gene expression in single cells

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Bioluminescence microscopy offers new avenues in live cell imaging. In contrast to fluorescence approaches, bioluminescence does not need excitation by light, as photon emission results from a chemical reaction:

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\text{Luciferin} + \text{ATP} + \text{O}_2 \rightarrow [\text{+Luciferase}] \rightarrow \text{Oxyluciferin} + \text{AMP} + \text{CO}_2 + \text{LIGHT}
\]

In comparison to fluorescence, bioluminescence has two major advantages: 1) Absence of phototoxicity which is especially valuable for long-term recording experiments. 2) Additionally results are highly specific and quantifiable. In distinction to fluorescence microscopy where autofluorescence of specimens as well as reflections or contamination from the excitation light can contribute to signal intensities, bioluminescence signals correspond in a one-to-one fashion to molecular events.

We have previously shown that cultured cells contain autonomous and self-sustained clocks using long-time fluorescence live cell imaging [1]. In later experiments we wanted to test robustness of circadian rhythms against changes in temperature or global transcription rates. For these experiments fluorescence microscopy could not be used: As temperature changes and drug treatments were needed, cells did not tolerate any additional phototoxic stress for more than a few hours. In contrast by using bioluminescence time-lapse microscopy circadian gene expression could be monitored and quantified over several days. Our results demonstrated that mammalian circadian oscillators are resilient to significant reductions in RNA polymerase II-dependent transcription and to temperature changes [2].

In a recent paper we showed that bioluminescence microscopy can be equally useful to precisely quantify short-time events [3]. In both prokaryotes and eukaryotes, transcription has been described as being temporally discontinuous, most genes being active mainly during short activity windows interspersed by silent periods. To characterize this in more detail transcription rates needed to be monitored at high temporal resolution in real time in single cells. This was done by first establishing various cell lines expressing a short-lived luciferase protein from an unstable mRNA. Using high camera binning (4x4 pixels) and photon counting mode we could then record and quantify transcription levels for up to 72 hours with a time resolution of five minutes. This allowed for the first time to precisely characterize and describe transcriptional kinetics of endogenous mammalian genes and led to the conclusion that mammalian genes are transcribed with widely different bursting rates having each its characteristic kinetics signature [3].

Currently we are working on improving ways to do two-color bioluminescence microscopy and to combine bioluminescence with fluorescence imaging.

REFERENCES: