Removing physiological motion from intravital and clinical fluorescence imaging data

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Intravital microscopy can provide unique insights into the function of biological processes in a native context. However, physiological motion caused by peristalsis, respiration and the heartbeat can present a significant challenge, particularly for functional readouts such as fluorescence lifetime imaging (FLIM) which require longer acquisition times to obtain a quantitative readout. This can prevent functional FLIM-FRET imaging in deep organs. We present and benchmark a versatile open source software tool, *Galene*, for image-based correction of sample motion blurring in both time resolved and conventional laser scanning fluorescence microscopy data in two and three dimensions. We show that *Galene* is able to resolve intravital FLIM-FRET, hyperspectral and image correlation spectroscopy images of intra-abdominal organs in murine models and NAD(P)H autofluorescence imaging of human dermal tissue subject to a wide range of physiological motions. For example, Figure 1 shows FLIM-FRET imaging of an intestinal crypt through an optical window using a transgenic Rac1 biosensor mouse. Peristaltic motion during acquisition leads to significant blurring of the crypt which can be corrected using *Galene*. We use the tool to image cancer cells expressing a Src FRET biosensor in an intrasplenic model of pancreatic cancer metastasis through an optical window. We reveal the dynamics of Src activation during adhesion and observe a significant delay in Src activity after priming with Fasudil at early time points, providing a potential mechanism for the significant reduction in metastasis observed in earlier end point experiments. The extensive physiological motion observed in the liver means that it would be impractical to acquire this data without correction. We thus provide a new tool to allow researchers to obtain insight into the earliest event in the metastatic cascade and as such a new avenue to insight into a critical event driving cancer colonisation for pancreatic cancer and more broadly other metastatic cancer types. We show that the software may be used in a broad range of intravital imaging experiments and imaging modalities and so enable functional imaging in situations where a stable imaging platform is not always possible.

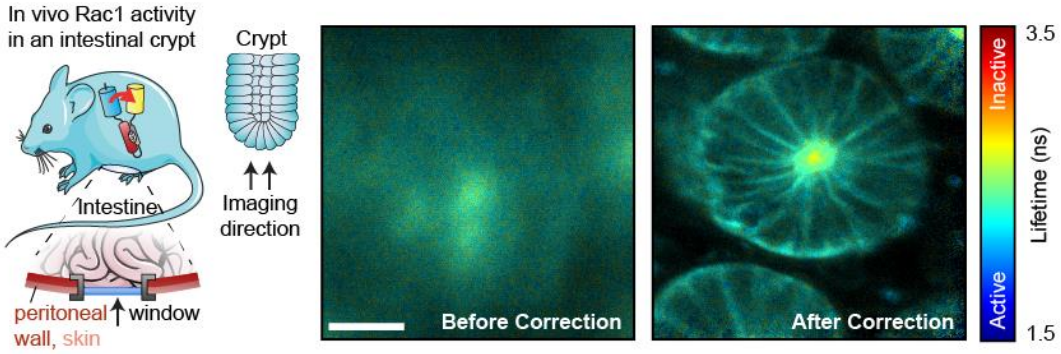

Figure 1. FLIM of intestinal crypts from the Rac1-FRET biosensor mouse in vivo using an abdominal titanium imaging window (left). Example intensity merged lifetime images (middle) before and (right) after motion compensation. White scale bar, 50 µm.

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