Method and software for model-based artifact correction in time-lapse fluorescence microscopy images

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Overview

Time-lapse microscopy (TLM) is a widely used method for studying dynamic biochemical and morphological responses in biological systems. Fluorescence TLM is usually implemented in wide-field imaging mode to minimise photobleaching and allow relatively high temporal resolution. Unfortunately, wide-field fluorescence images often suffer from hardware-dependent intensity artifacts that degrade the quality of the image data. These artifacts may be image coordinate-dependent, time-dependent, or both. They are usually poorly controlled and often unavoidable (e.g., photobleaching).

Starting from a general fluorescence image formation model, we have designed several artifact correction methods addressing the most common practical cases. These approaches require the acquisition of reference image data as part of the experimental protocol, although sometimes it may be possible to derive satisfactory corrections from sample image data itself. The proposed techniques may correct images for XY-dependent artifacts, including excitation non-uniformity, optical vignetting, and stray light, as well as time-dependent artifacts, e.g., arising from excitation light source power variations during a time course and inherent photobleaching of cell culture medium.

We have implemented these methods in a convenient open-source, cross-platform MATLAB software tool with GUI, capable of performing artifact compensation, image formatting, and parallelized batch processing of multi-channel TLM data of different types, aiming to make TLM image data more amenable for further quantitative analysis.

Problem formulation

Several approaches aiming to compensate intensity artifacts in wide-field fluorescence microscopy images have been presented that typically derive additive and multiplicative correction images from sample image data to remove non-uniform background [1,2]. Such methods are of limited practical applicability for TLM as they do not take into account temporal artifacts, do not use intensity models in a flexible way, and do not conveniently support multi-channel imaging. Here we aim to address these drawbacks and provide software tools to explore the impact of different artifact models and to support a range of image manipulations including XY downsampling, and selection and re-ordering of image channels in the output.

Artifacts correction method

Fluorescence microscopy image formation with artifacts:

We start with the following expression for the measured intensity $I=I(x,y;t)$ of an image registered at the camera plane of the microscope in a time course:

$$I = E \cdot p_{1,xy} f_1(t)[O + b \cdot p_{2,xy} f_2(t)] + offset \qquad (1)$$

where
- E is a constant proportional to the excitation power at the moment $t=0$ (first frame in the beginning of the experiment)
- $O(x,y;t)$ is the brightness of imaged fluorescing objects of interest. The data $EO(x,y;t)$ would be acquired if the imaging system was free of artifacts. The brightness $O(x,y;t)$ is understood as the product of the fluorophores' quantum yield, molar excitation extinction coefficient and concentration
- $offset$ is the coordinate- and time-independent camera offset (which includes dark noise)
- $p_{1(2),xy}$ are time-independent, spatially varying multiplicative (additive) artifacts across FOV (assuming smooth XY-dependence)
- $f_{1(2)}(t)$ are the spatially independent, time-dependent artifacts in illumination (emission)
- b is a constant proportional to the brightness of the additive artifacts (see below)

Equation (1) is similar to the model used in [1] but with factorized artifact terms. It assumes:
- functions $f(t)$ are normalized by their value at $t=0$, so as $f(0)=1$,
- images p_{xy} are normalized by the maximum using the operation $p_{xy} = p_{xy}/max_{[XY]}(p_{xy})$

Thus, the constant b is the maximum brightness (over XY plane) of additive artifacts at $t=0$

The (multiplicative) artifact image $p_{1,xy}$ may describe phenomena such as excitation non-uniformity and optical vignetting, whereas the (additive) image $p_{2,xy}$ may represent stray light or the sample medium's fluorescence.

Similarly, the function $f_1(t)$ can describe variations in excitation power during time course, while the $f_2(t)$ can describe the sample medium's photobleaching.

We will further assume that the camera offset is known and has been subtracted (i.e. $offset=0$).

We consider 4 practical specific cases of (1) where one of the p_{xy} images and one of the $f(t)$ functions are both set to 1, and the offset is set to 0:

$$
\begin{aligned}
&a) \quad I = E \cdot [O + b \cdot p_{xy} f(t)], \quad \text{presuming } f_1(t)=1, p_{1,xy}=1 \\
&b) \quad I = E \cdot p_{xy}[O + b \cdot f(t)], \quad \text{............ } f_1(t)=1, p_{2,xy}=1 \\
&c) \quad I = E \cdot f(t)[O + b \cdot p_{xy}], \quad \text{............ } f_2(t)=1, p_{1,xy}=1 \\
&d) \quad I = E \cdot p_{xy} f(t)[O + b], \quad \text{............ } f_2(t)=1, p_{2,xy}=1
\end{aligned}
\qquad (2)
$$

Compensation technique:

When imaging the field of view (FOV) not containing any cells, but only the medium in which they are placed in the experiment, a time-lapse sequence of reference image data is recorded,

$I_{ref} = I_{ref}(x,y;t)$.

Since there are no fluorescent samples in the reference image data (i.e. $O(x,y;t)=0$), all expressions in (2) become:

$$I_{ref} = (Eb) \cdot p_{xy} f(t) \qquad (3)$$

Expression (3) allows the functions p_{xy} and $f(t)$ and the constant (Eb), to be estimated from only the reference image data I_{ref}, using simple image arithmetic and basic image processing tools. Presuming that the compensation objects p_{xy}, $f(t)$ and (Eb) are known, one can derive from (2) the following expressions for the sample brightness $O(x,y;t)$ scaled by the constant E:

$$
\begin{aligned}
&a) \quad EO = I - Eb \cdot p_{xy} f(t) \\
&b) \quad EO = I / p_{xy} - Eb \cdot f(t) \\
&c) \quad EO = I / f(t) - Eb \cdot p_{xy} \\
&d) \quad EO = I / [p_{xy} f(t)] - Eb
\end{aligned}
\qquad (4)
$$

Given the reference image data I_{ref}, these equations (4) can be applied to correct artifacts in TLM data using the ***MicroscopyImageFormatter*** software [3], which includes a flexible GUI.

References

1. Kask P., et al, Flat field correction for high-throughput imaging of fluorescent samples, (2016),J. Microscopy 263(3),328
2. Smith, K., et al, CIDRE: an illumination-correction method for optical microscopy, (2015) Nat. Meth.12, 404-406 (11)
3. "MicroscopyImageFormatter", as part of ***https://github.com/yalexand/ALYtools***

TLM images correction software:

The application [3] was programmed in MATLAB, utilising Bioformats I/O. Options to batch-process many multi-channel images, and to visualize corrected images, are implemented. This software is capable of deriving approximate correction objects from data if I_{ref} is not provided
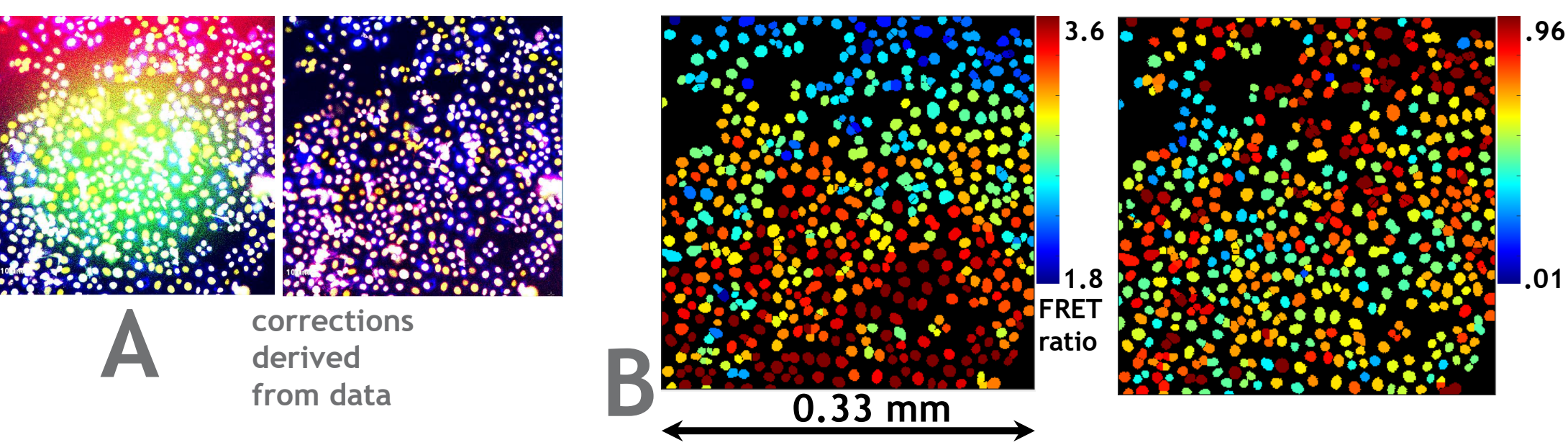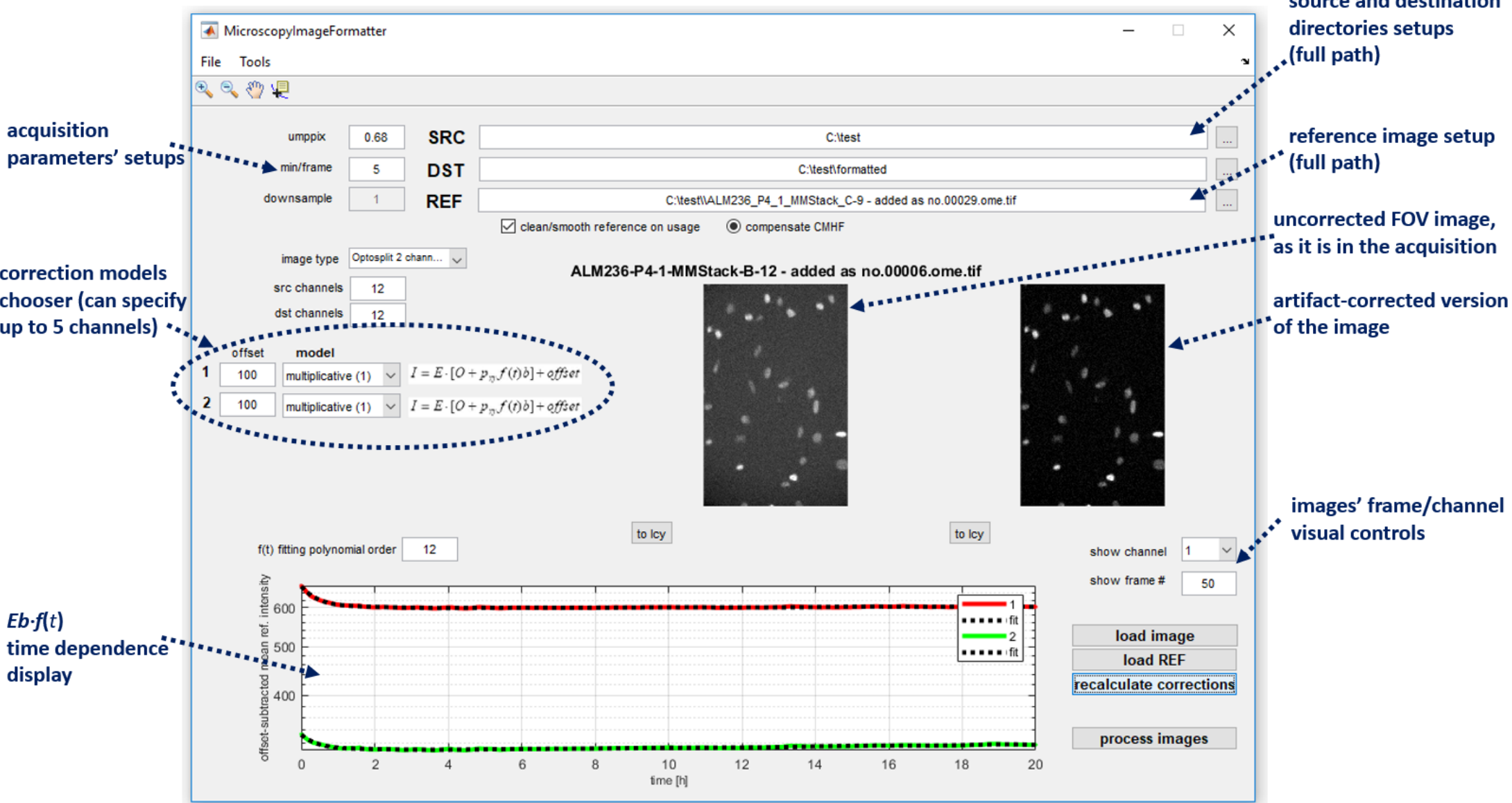
Late time-course image frame from a 3-color wide-field fluorescence TLM data set of PC9 cells (EGFR-mutant lung cancer) expressing CFP-YFP EKAREV-NLS FRET sensor for ERK activity (in nucleus), and FoxO1-mRuby2 KTR sensor for Akt activity (in cell body). Cells are cultured in RPMI. (A) intensity pseudo colour images with red donor/green acceptor and blue for cell body (B) FRET ratio maps following segmentation of cell nuclei. Left hand images in (A,B) are raw data and right hand images are corrected with the model 4b).
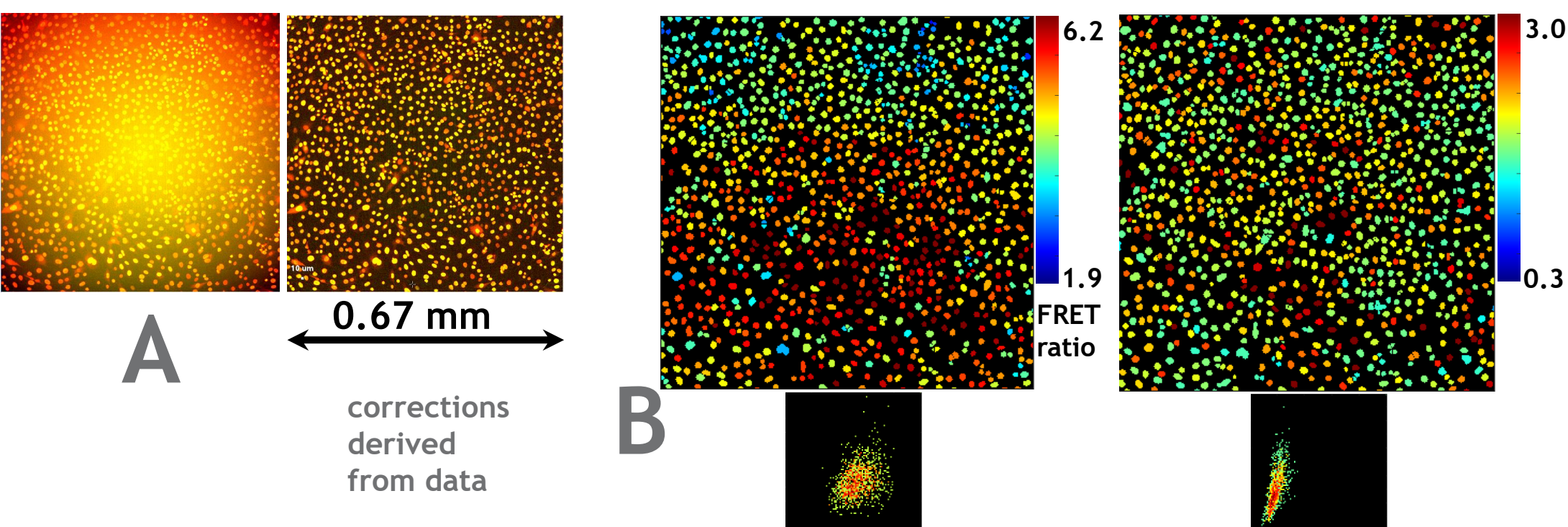
Late time-course frame from a 2-color wide-field fluorescence TLM data set of YT 123 cMyc cells stably transfected with the YPet-TurquoiseGL FRET pair labelling G-Actin in the nucleus compartment. (A) intensity pseudo colour images with red donor/green acceptor and (B) FRET ratio maps following segmentation of cell nuclei with corresponding 2D value-temporal variability histograms for nuclear tracks. Left hand images in (A,B) are raw data and right hand images are corrected with the model 4d). Histograms show that the correction can recover potentially meaningful signal correlations in the tracking data.
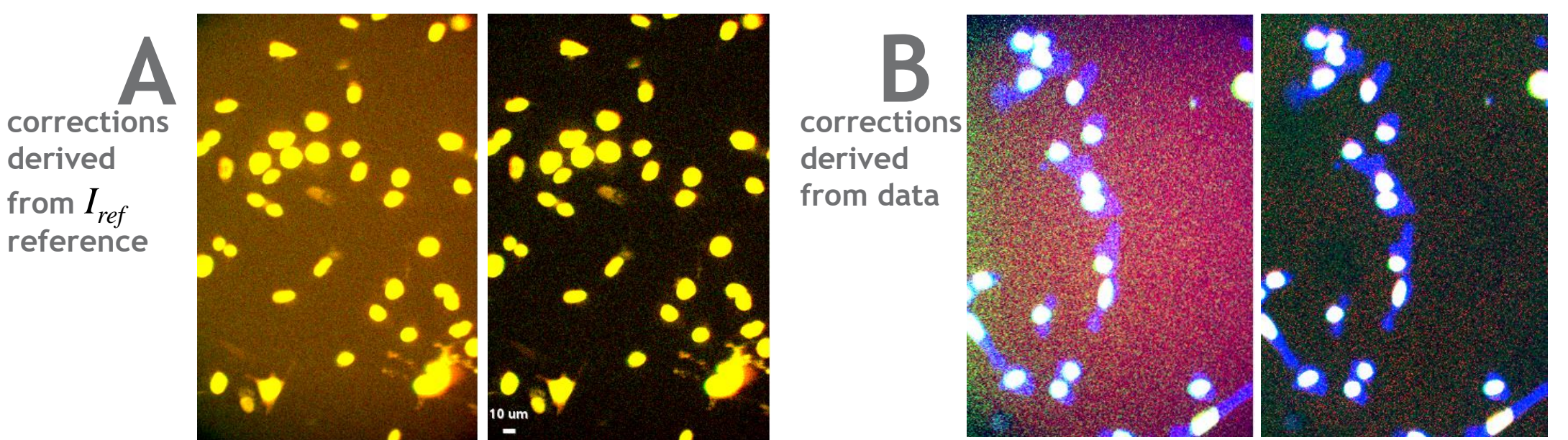

Image frames from 2- and 3-color FRET TLM screens of (A): H1975 cells (EGFR-mutant lung cancer) expressing CFP-YFP EKAREV-NLS sensor for ERK activity in nucleus and cultured in phenol red-free RPMI; (B) PC9 (small cell lung cancer) cells, expressing the same CFP-YFP sensor for pERK activity in nuclei, and pAKT-mRuby2 in cell body and cultured in RPMI.
(A) intensity pseudo colour images with red donor/green acceptor; (B), intensity pseudo colour images with red donor/green acceptor and blue for cell body. Left hand images in (A,B) are raw data and right hand images are corrected with the model 4b).