Updated 02/12/19

Text for Materials & Methods Sections

The descriptions below can be used in the materials & methods sections of publications to describe the imaging system you have been using. Please read carefully as this information can be used as a starting point.

IMPORTANT: There is no info about which objective is used here or the binning on the camera. You need to put this information in yourself!

Image analysis: Data were analysed using Image J 2.0 (Check your version) and 64bit Java8. Custom scripts and documentation of bespoke analysis can be found on the IGMM imaging Github https://github.com/igmm-imaging.

3D datasets were visualized and analysed using Imaris V9.1(Bitplane, Oxford Instruments, Oxfordshire, UK).

Quantiative analysis of Tissue pathology images was carried out using Definiens AG (Munich, Germany).

Limelight and Mac Red

For fluorescence 3 colour imaging with single emission filters:

Epifluorescent images were acquired using a Photometrics Coolsnap HQ2 CCD camera and a Zeiss Zeiss Axioplan II fluorescence microscope with Plan-neofluar objectives (Carl Zeiss, Cambridge, UK), a Mercury Halide fluorescent light source (Exfo Excite 120, Excelitas Technologies) and Chroma #83000 triple band pass filter set (Chroma Technology Corp., Rockingham, VT) with the excitation filters installed in a motorised filter wheel (Ludl Electronic Products, Hawthorne, NY). Image capture was performed using Micromanager (https://open-imaging.com/).

For fluorescence 4 colour imaging with single emission filters

Epifluorescent images were acquired using a Photometrics Coolsnap HQ2 CCD camera and (Photometrics Ltd, Tucson, AZ) mounted on a Zeiss Axioplan II fluorescence microscope with Plan-neofluar/apochromat objectives(Carl Zeiss, Cambridge, UK), a Mercury Halide fluorescent light source (Exfo Excite 120, Excelitas Technologies) and Chroma #89000ET four colour filter set (Chroma Technology Corp., Rockingham, VT). The single excitation and emission filters are installed in motorised filter wheels (Prior Scientific Instruments, Cambridge, UK). Image capture was performed using Micromanager (https://open-imaging.com/).

Brightfield colour imaging on MacRed

Brightfield images were acquired using a QImaging R6 colour CCD camera (Qimaging, Surrey, BC, Canada) mounted on a Zeiss Axioplan II fluorescence microscope with Plan-neofluar or Plan Apochromat objectives (Carl Zeiss, Cambridge, UK). Image capture was performed using Micromanager (https://open-imaging.com/).

Britemac

For fluorescence 3 colour imaging with single emission filters:

Epifluorescent images were acquired using a Photometrics Prime BSI CMOS camera and a Zeiss AxioImager M2 fluorescence microscope with Plan-Apochromat objectives (Carl Zeiss, Cambridge, UK) , a Mercury Halide fluorescent light source (Exfo Excite 120, Excelitas Technologies) and Chroma #89014ET three colour filter set (Chroma Technology Corp., Rockingham, VT) The single excitation and emission filters are installed in motorised filter wheels (Prior Scientific Instruments, Cambridge, UK). Image capture was performed using Micromanager (https://open-imaging.com/).

For four colour fluorescence imaging with single emission filters:

Epifluorescent images were acquired using a Photometrics Prime BSI CMOS camera and a Zeiss AxioImager M2 fluorescence microscope with Plan- Apochromat objectives (Carl Zeiss, Cambridge, UK) , a Mercury Halide fluorescent light source (Exfo Excite 120, Excelitas Technologies) and Chroma #89000ET four colour filter set (Chroma Technology Corp., Rockingham, VT) with the single excitation and emission filters installed in motorised filter wheels (Prior Scientific Instruments, Cambridge, UK). Image capture was performed using Micromanager (https://open-imaging.com/).

Granny (or New Granny from autumn 2015)

For 3D imaging of fixed material:

3 colour imaging with single emission filters

Epifluorescent images were acquired using a Photometrics Coolsnap HQ2 CCD camera and a Zeiss AxioImager A1 fluorescence microscope with a Plan Apochromat 100x 1.4NA objective, a Nikon Intensilight Mercury based light source (Nikon UK Ltd, Kingston-on-Thames, UK) and either Chroma #89014ET (3 colour) or #89000ET (4 coour) single excitation and emission filters (Chroma Technology Corp., Rockingham, VT) with the excitation and emission filters installed in Prior motorised filter wheels. A piezoelectrically driven objective mount (PIFOC model P-721, Physik Instrumente GmbH & Co, Karlsruhe) was used to control movement in the z dimension. Hardware

control, image capture and analysis were performed using Nikon Nis-Elements software (Nikon UK Ltd, Kingston-on-Thames, UK).

Deconvolution of 3D data:

Images were deconvolved using a calculated PSF in Volocity (Perkinelmer Inc, Waltham MA). **You need to specify how you did this either fast or iterative**

Santana

The Microscope comprises a Zeiss Axiovert 200 fluorescence microscope (Carl Zeiss UK, Cambridge, UK), X-Cite ExFo 120 Mercury Halide (Exfo X-cite 120, Excelitas Technologies) fluorescent source (Previously a Lambda LS 300W Xenon source) with liquid light guide and 10-position excitation, neutral density and emission filterwheels (Sutter Instrument, Novato, CA), ASI PZ2000 3-axis XYZ stage with integrated piezo Z-drive (Applied Scientific Instrumentation, Eugene, OR), Retiga R1 CCD camera (Qimaging, Surrey, BC, Canada) The filter wheels are populated with #86000 Sedat quad set and #89903 ET BV421/BV480/AF488/AF568/AF647 quinta set. Legacy sets include #86002v2 CFP/YFP set and #86007 EGFP/DsRed) set (Chroma Technology Corp., Rockingham, VT). Image capture was performed using Micromanager 1.4 (https://open-imaging.com/).

Calvados

Images were acquired using **X Lens** on a Zeiss Axio-Observer Z1 inverted microscope (Carl Zeiss UK, Cambridge, UK), with a ASI MS-2000 XY stage (Applied Scientific Instrumentation, Eugene, OR). Samples were illuminated using Brightfield or a Lumencor Spectra X LED light source (Lumencor Inc, Beaverton, OR) complete with Chroma #89000ET single excitation and emission filters (Chroma Technology Corp., Rockingham, VT) and acquired on a Retiga6000 CCD camera (Qimaging, Surrey, BC, Canada) or a Evolve EMCCD camera (Photometics, Photometrics, Tucson, AZ). Cells were maintained at 37ºC using a custom made environmental enclosure (MRC Human Genetics Unit, Edinburgh, UK) A 5% CO2 atmosphere was maintained by a Okolabs chamber(Okolabs Pozzuoli NA, Italy). Image capture was performed using Micromanager (https://open-imaging.com/).

Cox + Red Royal

The imaging system comprises a Leica MZFLIII fluorescence stereo microscope with 0.5x, 0.63x, 1x, 1.6x objectives and Leica GFP1, GFP2, UV, B, G filters (select as appropriate) fitted with a Qimaging Retiga Exi CCD camera (Qimaging, Surrey, BC, Canada). Image capture was performed using Micromanager (https://open-imaging.com/).

Nikon Macroscope AZ100

The imaging system comprises a Nikon AZ100 macroscope with 0.5x, 1x, 2x, 4x and 5x objectives, Intensilight 130W Hg light source and Nikon UV, G or B filter cubes (Nikon UK Ltd, Kingston-on-Thames, UK). For fluorescence work a Qimaging Retiga EXi camera is used and for colour brightfield work a Qimaging Micropublisher 5 cooled colour camera (Qimaging, Qimaging, Surrey, BC, Canada). Image capture was performed using Micromanager (https://open-imaging.com/).

Dragonfly

Images were acquired using a X lens on the multimodal Imaging Platform Dragonfly (Andor technologies, Belfast UK) equipped with 405, 445, 488, 514, 561, 640 and 680nm lasers built on a Nikon Eclipse Ti-E inverted microscope body with Perfect focus system (Nikon Instruments, Japan). Data were collected in Spinning Disk 40µm pinhole/ Spinning Disk 25µm pinhole /Widefield/ Total Internal Reflection Fluorescence (delete as appropriate) mode on the iXon 888 EMCCD / Zyla 4.2 sCMOS camera using a Bin of X and frame averaging of X using Andor Fusion acquisition software.

If required: Z stacks were collected using the Nikon TiE focus drive or Mad City Labs Piezo (delete as appropriate), Multiple positions were collected using a Sigma-Koki Stage (Nikon Instruments Japan)

Data were visualized using Imaris v9.0 or Fiji.

Nikon A1R (Butterfly) & A1 (Firefly) confocal microscopes

Images were acquired on a Nikon Confocal A1R or A1 confocal microscope using a ??X ??NA objective. The microscope comprises of a Nikon Eclipse TiE inverted microscope with Perfect Focus System and is equipped with 405nm diode, 457/488/514nm Multiline Argon, 561nm DPSS and 638nm diode lasers. Galvanometer or resonant scanning mirrors can be chosen depending on the application (Resonant scanner on Butterfly only) (delete as appropriate). Detection is via four Photomultiplier tubes (2x standard Photomultiplier tubes and 2x GaAsP PMTs) or a 32PMT spectral detector (delete as appropriate) (spectral detector on Butterfly only). Data were acquired using NIS Elements AR software (Nikon Instruments Europe, Netherlands). Images were scanned at (State scan size and zoom and frame averaging is used).

For live cell work, environmental control of the cultured cells is maintained during imaging with a Solent Scientific incubation chamber incorporating temperature and humidified CO2 control (Solent Scientific Ltd., Segensworth, UK).

Leica SP5 (Bumblebee)

Images were acquired using a Leica SP5 confocal (Leica Microsystems Milton Keynes UK) using LAS-AF software. The microscope is equipped with 405nm diode, Argon and, 561 and 648nm laser lines, three Photomultiplier tubes and one HyD GaSP detector. Images were scanned using a ??X ??NA objective. (State scan size and zoom and frame averaging is used).

FCS images were acquired using LAS (Leica Microsystems, Germany) and processed in Symphotime 64 software using a PicoHarp 300 TCSPC module and APD detectors (Picoquant). Data collected were an average of x positions integrated over X time.

Nikon N-SIM

Super-resolution images were acquired using structured illumination microscopy.. Samples were prepared on high precision cover-glass (Zeiss, Germany). 3D SIM images were acquired on a N-SIM (Nikon Instruments, UK) using a 100x 1.49NA lens and refractive index matched immersion oil (Nikon Instruments). Samples were imaged using a Nikon Plan Apo TIRF objective (NA 1.49, oil immersion) and an Andor DU-897X-5254 camera using 405, 488, 561 and 640nm laser lines (**delete as appropriate**). Z-step size for Z stacks was set to 0.120 um as required by manufacturers software. For each focal plane, 15 images (5 phases, 3 angles) were captured with the NIS-Elements software. SIM image processing, reconstruction and analysis were carried out using the N-SIM module of the NIS-Element Advanced Research software. Images were checked for artefacts using the SIMcheck software (http://www.micron.ox.ac.uk/software/SIMCheck.php). Images were reconstructed using NiS Elements software v4.6 (Nikon Instruments, Japan) from a z stack comprising of no less than 1um of optical sections. In all SIM image reconstructions the Wiener and Apodization filter parameters were kept constant (Or say what you did if these varied).

Any reviewer questions about how or why the SIM was done the way it was can be rebutted using this citation:

http://www.cell.com/biophysj/abstract/S0006-3495%2808%2970360-6

since we effectively follow the same protocol and the first author developed SIM.

Please acknowledge ESRIC in the acknowledgements section and the member of facility staff who assisted you.

Thunder (Nikon N-STORM)

The microscope comprises of a Nikon Eclipse Ti-E inverted microscope with laser TIRF illuminator (Nikon UK Ltd, Kingston Upon Thames, UK). A CFI SR HP Apo TIRF 100x objective lens. Laser light is provided via a Nikon LU-NV laser bed with 405, 488, 561, 640nm laser lines. Widefield illumination is provided by a CoolLED PE-4000 LED light source (CoolLED Ltd, Andover, UK). Images were acquired with an Andor iXon 897 EMCCD camera (Andor technologies, Belfast UK). **Please acknowledge ESRIC in the acknowledgements section the member of facility staff who assisted you.**

Thunder (Widefield)

The microscope comprises of a Nikon Eclipse Ti-E inverted microscope with laser TIRF illuminator (Nikon UK Ltd, Kingston Upon Thames, UK). A CFI SR HP Apo TIRF 100x objective lens. Laser light is provided via a Nikon LU-NV laser bed with 405, 488, 561, 640nm laser lines. Widefield illumination is provided by a CoolLED PE-4000 LED light source (CoolLED Ltd, Andover, UK) combined with either the Semrock Brightline LED-DA/FI/TR/Cy5-4X4M-B (Sedat), LED-CFP/YFP/mCherry-3X-A (Pinkel) filter sets or Nikon single band pass filter cubes for DAPI, FITC & Texas Red. Images were acquired with an Andor iXon 897 EMCCD camera (Andor technologies, Belfast UK) or a Photometrics Prime BSI sCMOS camera (Teledyne Photometrics, 3440 E. Britannia Drive, Suite 100, Tucson, AZ 85706). Conditional imaging was setup and controlled through Nikon Jobs. Please acknowledge Matt in the acknowledgements section if he programmed the 'job' for you.