MesoTIRF: Total Internal Reflection Fluorescence across a 4.4 mm x 3.0 mm Field of View

Shannan Foylan [1], Michael Shaw [2,3], Gail McConnell [1]

[1] Department of Physics, SUPA, University of Strathclyde, Glasgow, UK, [2] Department of Chemical and Biological Sciences, National Physical Laboratory, Teddington, UK
[3] Department of Computer Science, University College London, London, UK

shannan.foylan@strath.ac.uk
@shannan_foylan

Motivation

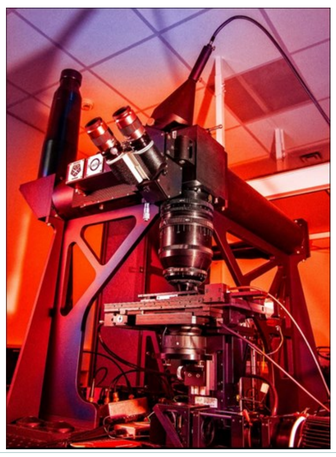
Total Internal Reflection Fluorescence microscopy:

- Resolve detail below the depth of field (DoF) of imaging objective
- Contrast/SNR improvement over conventional widefield fluorescence microscopy
- Elucidate detail on membrane biophysics [1, 2]
- Illuminator for many SMLM techniques [3, 4]
- TIRF objectives restrict lateral imaging field to < 0.3 mm^2
- Resolution/contrast improvement only visible over a handful of cells simultaneously

Our solution: MesoTIRF

Prism-based TIRF modality across the 4.4 mm x 3.0 mm FOV of the **Mesolens** [5]

- N.A. = 0.47, r_{lat} = 700 nm
- Optical section << DoF
- TIRF benefit over 1000 mammalian cells in a single image

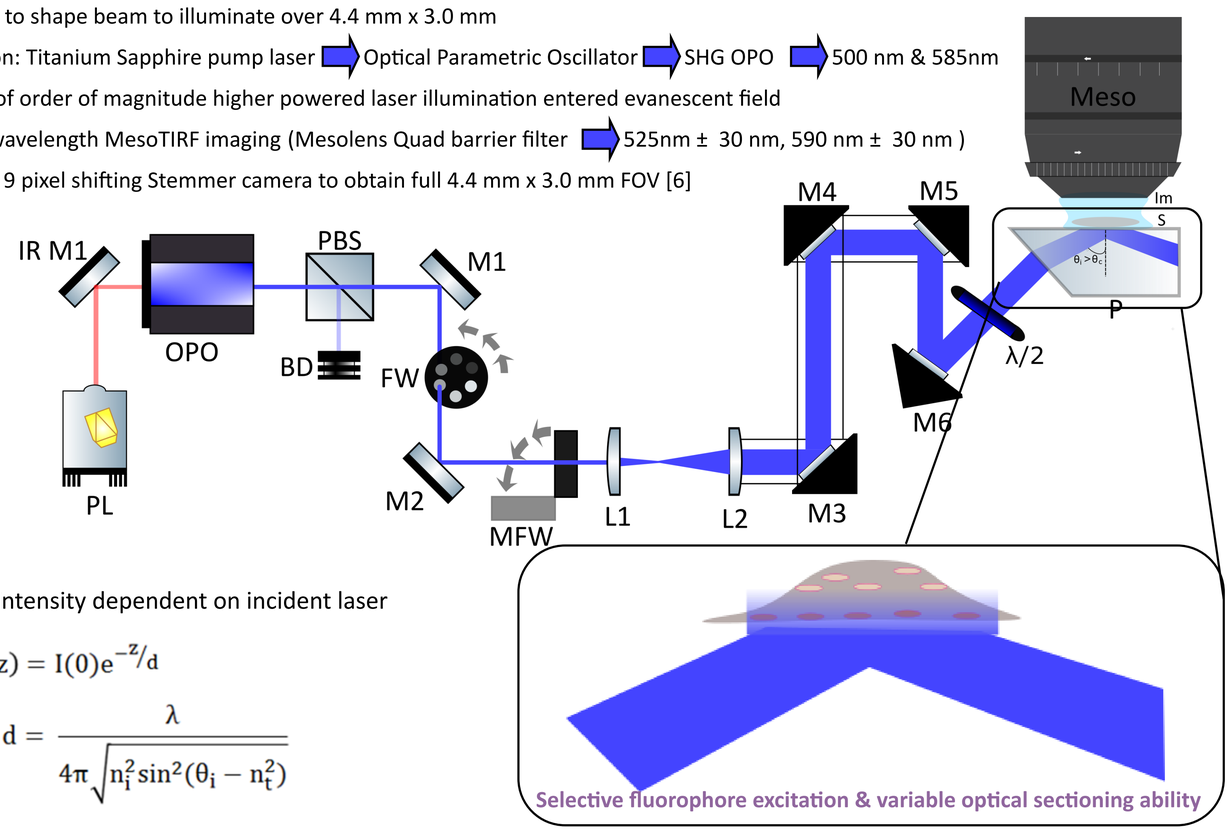


Optical set-up

Gaussian optics used to shape beam to illuminate over 4.4 mm x 3.0 mm

MesoTIRF illumination: Titanium Sapphire pump laser → Optical Parametric Oscillator → SHG OPO → 500 nm & 585nm

- Ensure 1-10 mW of order of magnitude higher powered laser illumination entered evanescent field
- Allows for multi-wavelength MesoTIRF imaging (Mesolens Quad barrier filter → 525nm ± 30 nm, 590 nm ± 30 nm)
- (Not pictured) 9 x 9 pixel shifting Stemmer camera to obtain full 4.4 mm x 3.0 mm FOV [6]



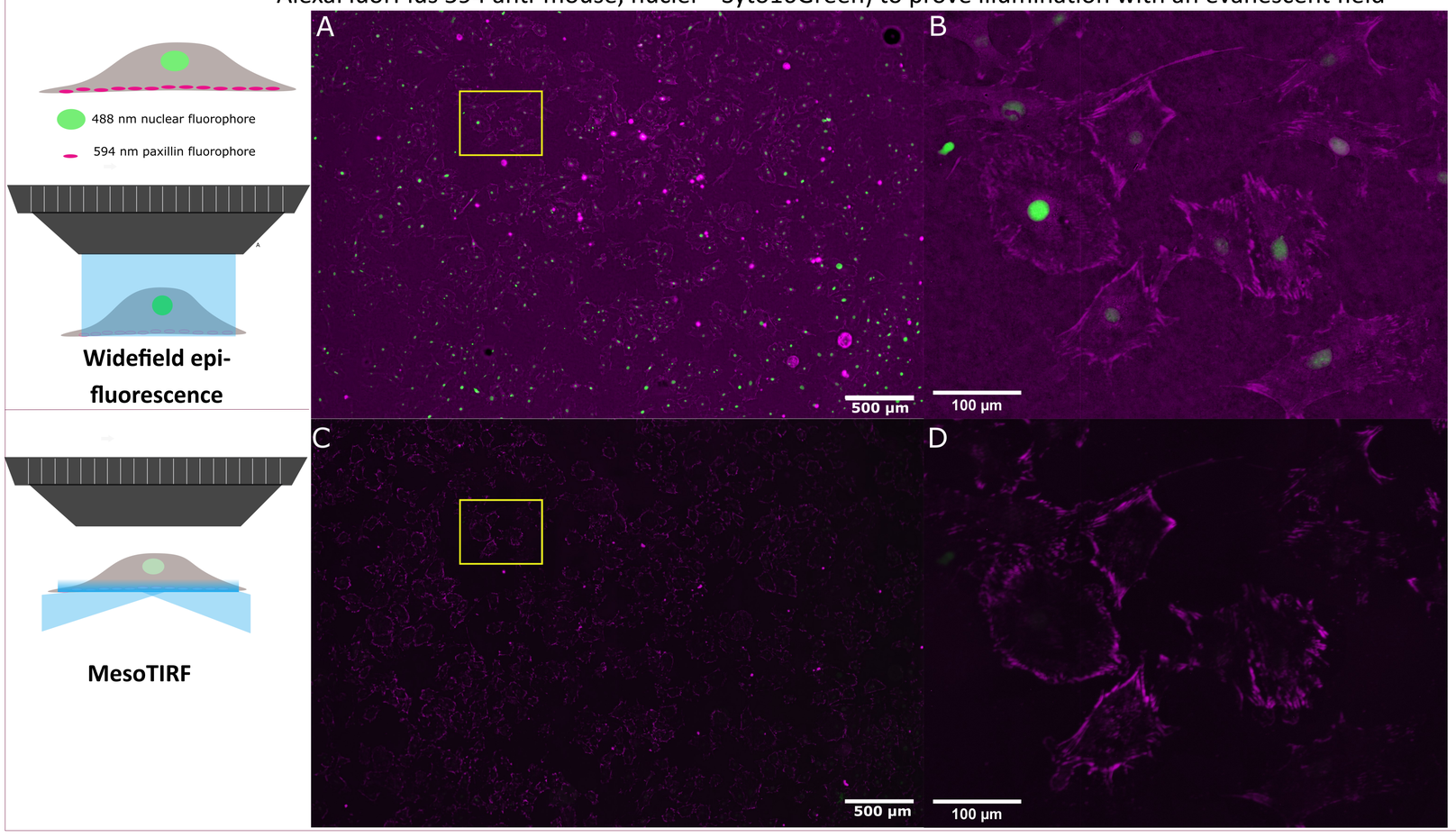
Illumination depth & intensity dependent on incident laser properties:

$$I(z) = I(0)e^{-z/d}$$

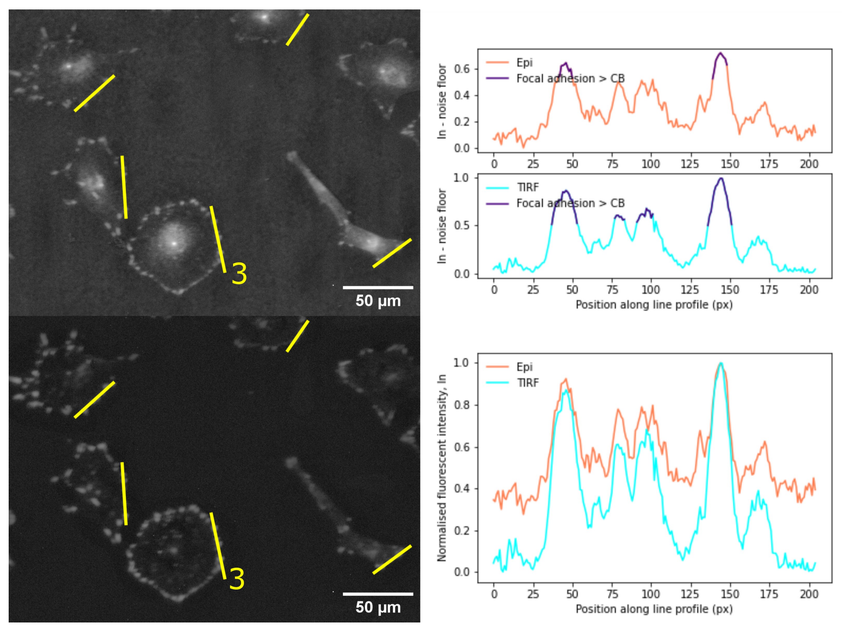
$$d = \frac{\lambda}{4\pi\sqrt{n_i^2 \sin^2(\theta_i - n_t^2)}}$$

Results

We use a dual labelled fixed cell specimen (focal adhesions: paxillin mouse anti-human primary—AlexaFluorPlus 594 anti-mouse, nuclei—Syto16Green) to prove illumination with an evanescent field

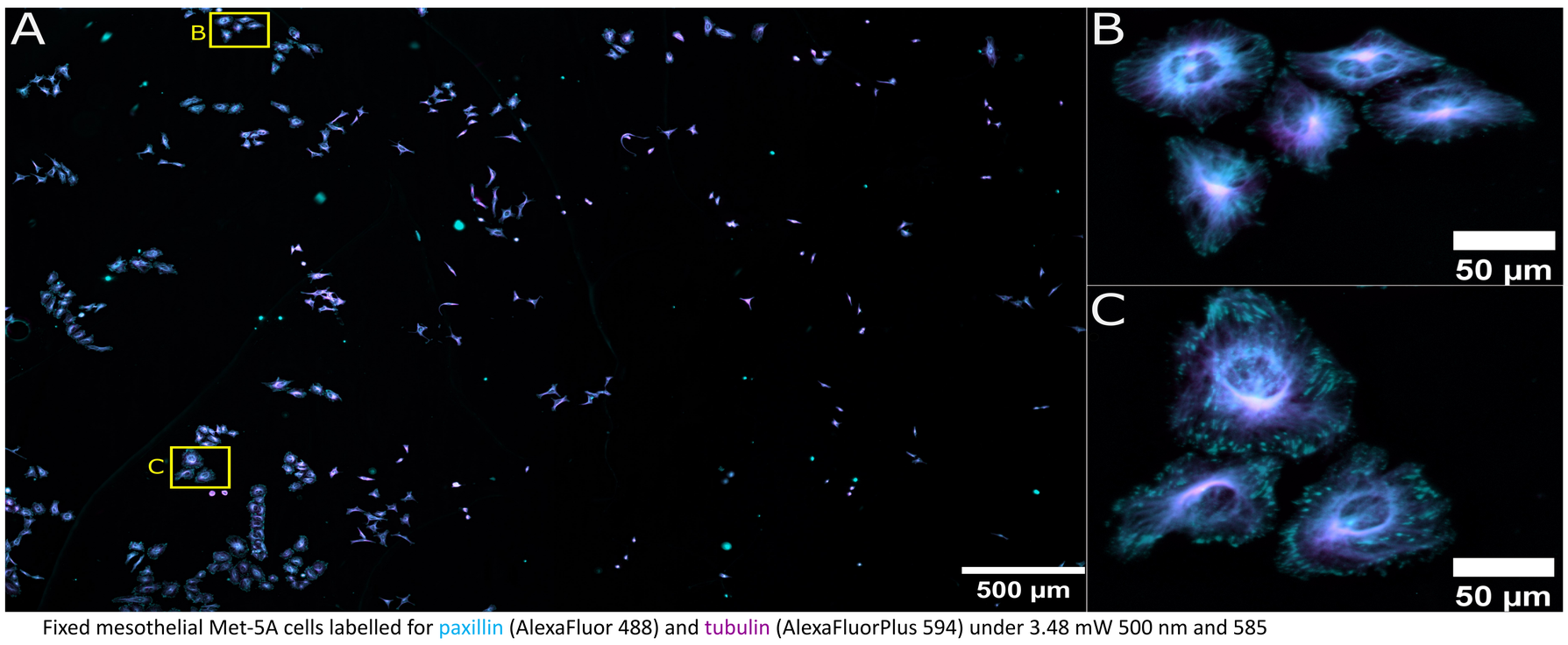


Labelled focal adhesions were used to compare contrast improvement when switching from widefield epi-fluorescence to MesoTIRF:



MesoTIRF is producing a characteristic evanescent field and we see improvement synonymous with TIRF imaging across 4.4 mm x 3.0 mm FOV

Multi-λ MesoTIRF



Fixed mesothelial Met-5A cells labelled for paxillin (AlexaFluor 488) and tubulin (AlexaFluorPlus 594) under 3.48 mW 500 nm and 585 nm TIRF illumination respectively. A: Mesolens full FOV, B, C: highlighted regions of interest

MesoTIRF at a glance

- 4.4 mm x 3.0 mm lateral imaging field
- Optical section thickness < Mesolens DoF
- 1.85 X improvement in signal-background ratio versus widefield epi-fluorescence
- Up to 5X fold reduction in non-specific background in MesoTIRF versus widefield epi-fluorescence
- 500 nm and 585 nm laser lines available for multi-colour MesoTIRF imaging, only limited by fluorescent barrier filters in Mesolens
- Suitable for imaging live samples at room temperature in water immersion

References

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