TartanSW: Filling the Information Gap in Standing Wave Microscopy

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Introduction

Widefield standing wave microscopy has been shown to provide axial resolutions below 100 nm that can be acquired at up to 100 frames per second with the only change to the imaging setup being the replacement of a standard microscope slide with a first surface reflector [1,2].

However, because this technique makes use of the interaction between a fluorescent specimen and the antinodal planes of an optical standing wave to achieve axial super-resolution the nodal plane contributions result in ~ 50 % of the specimen not being imaged.

We present a method called TartanSW which makes use of standing waves of different wavelengths to shift the antinodal plane axial locations and hence reduce the amount of missing axial information in the image.

Principal of TartanSW Microscopy

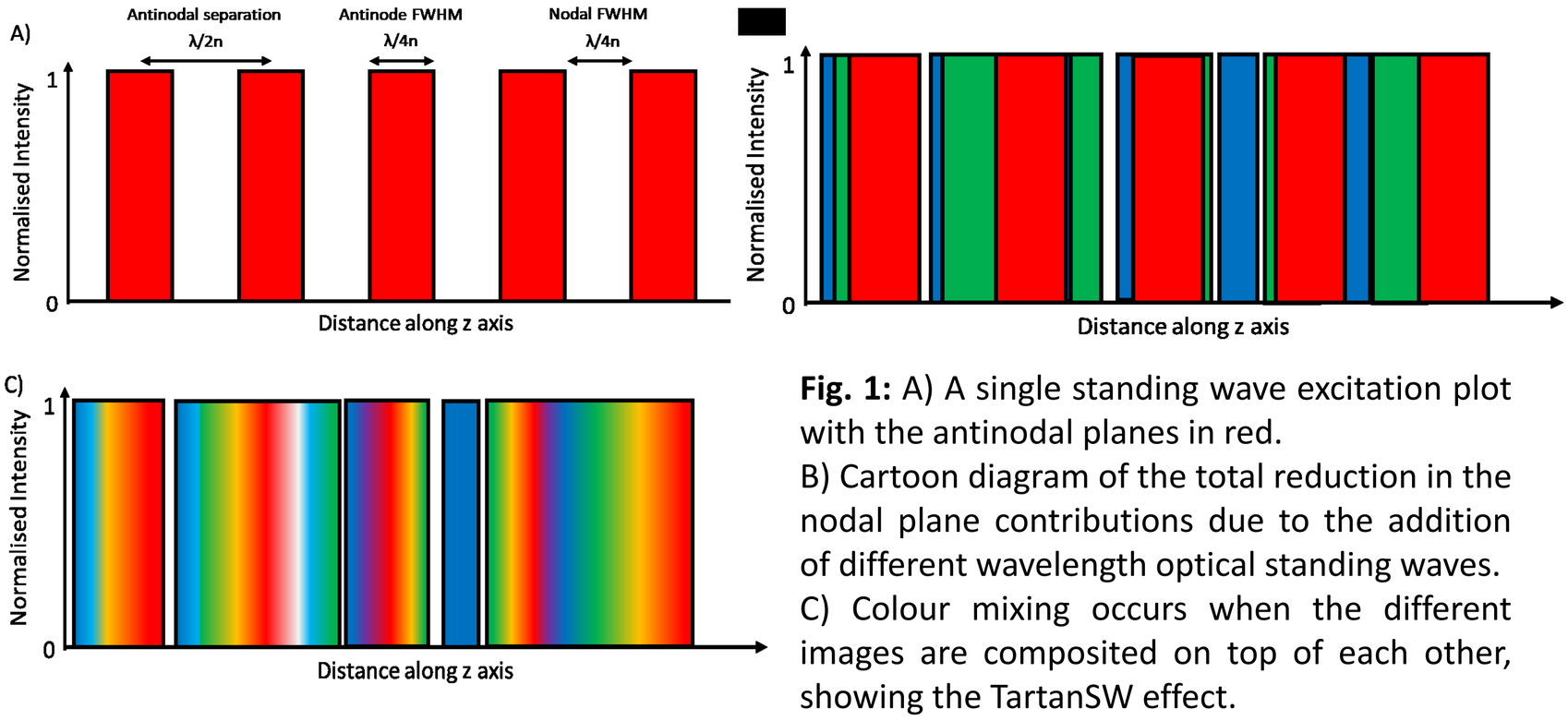

Fig. 1: A) A single standing wave excitation plot with the antinodal planes in red.
B) Cartoon diagram of the total reduction in the nodal plane contributions due to the addition of different wavelength optical standing waves.
C) Colour mixing occurs when the different images are composited on top of each other, showing the TartanSW effect.

TartanSW Experimental Setup

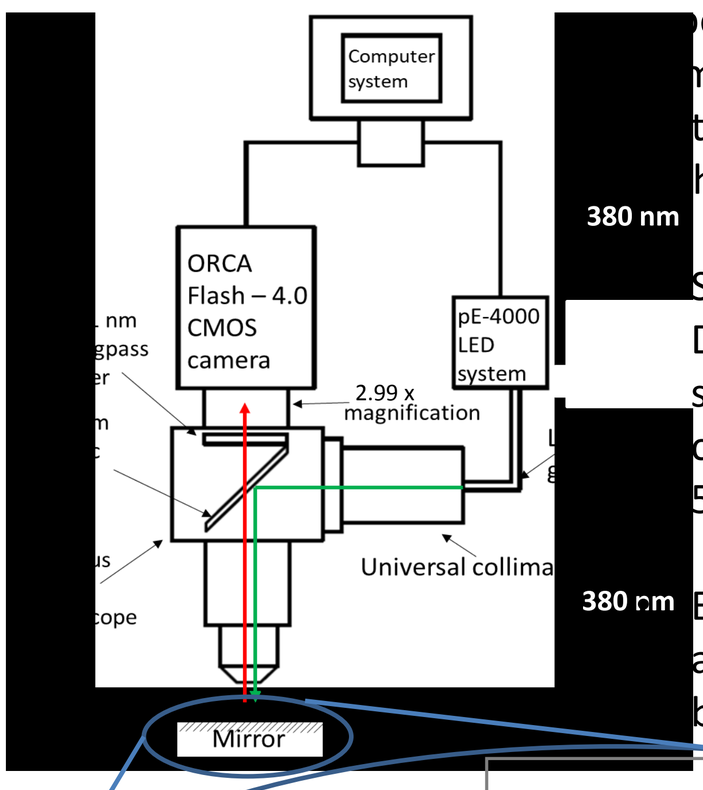

Fig. 2: Schematic diagram of experimental set up and demonstration of how TartanSW structure interacts with specimens.

Specimens are stained with DiI and excitation is supplied sequentially by a custom LED system at 550, 525 and 490 nm.

Exposure times < 100 ms are used with a camera binning n = 2

Information Gap Reduction Using TartanSW

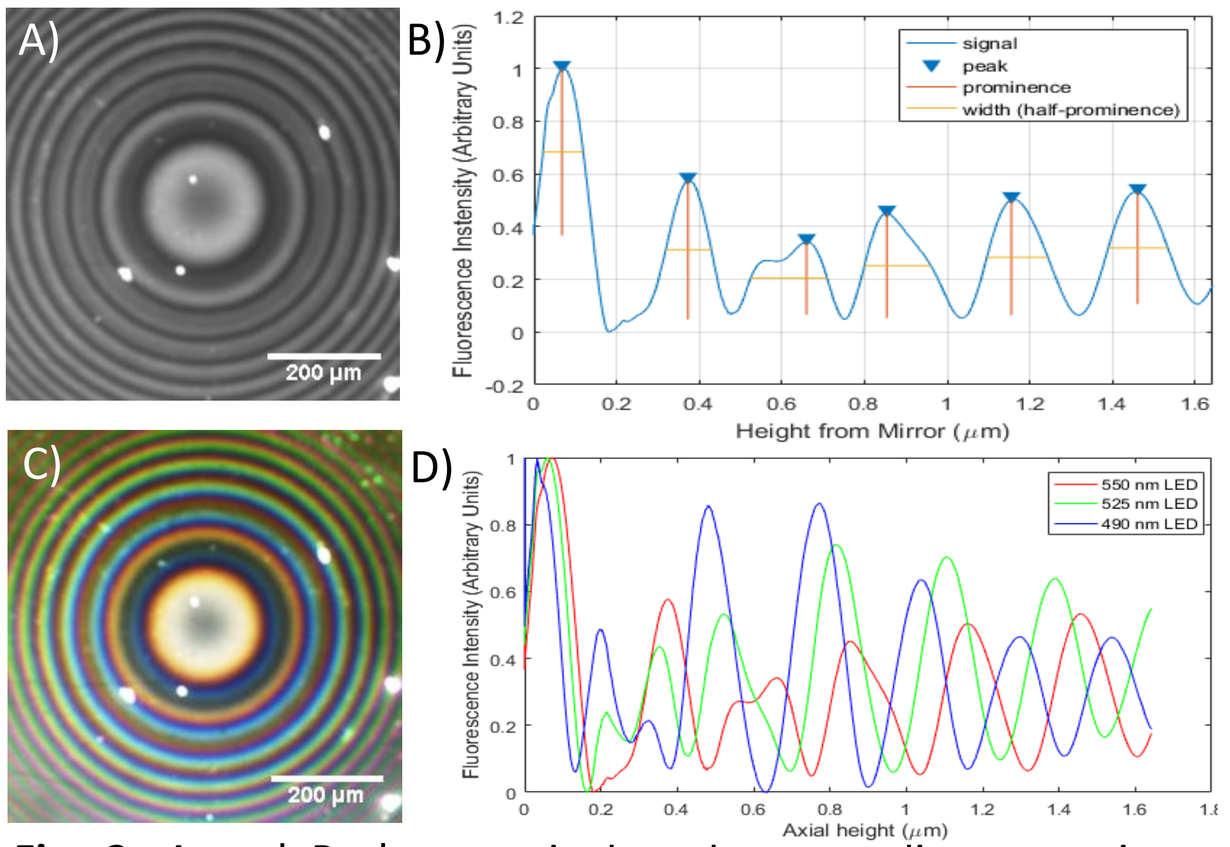

Fig. 3: A and B show a single colour standing wave image obtained using a 10x/0.4 dry objective lens and radially averaged intensity plot of a fluorescently coated lens specimen obtained as described previously [2]. C and D show TartanSW and radially averaged intensity plot of the same fluorescent lens specimen. **TartanSW allows for 36 % more information about the specimen to be obtained.**

TartanSW Of Live MCF-7 Cells

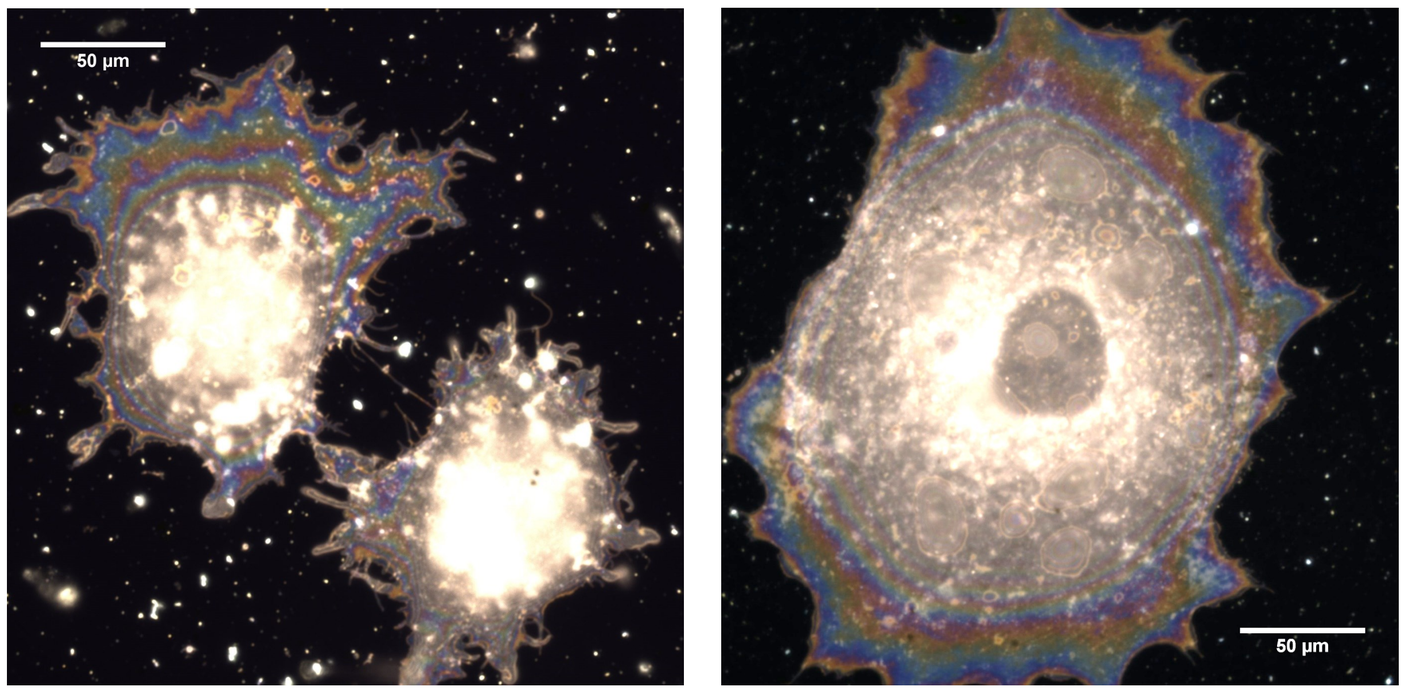

Fig. 4: TartanSW images of the membrane of live MCF-7 cells labelled with DiI (5.35 µM) imaged in Hepes buffered solution using a 60x/0.75 water dipping objective lens. **TartanSW gives widefield axial resolutions below 90 nm with a greater reduction in missing information in live cell specimens.**

Conclusions and Future Work

- **Widefield TartanSW has been demonstrated to reduce the information gap present in standing wave microscopy by up to 36 % with only the addition of two additional excitation wavelengths.**
- **Axial resolutions below 90 nm are retained and by using rapid switching LED illumination high frame rate acquisition can also be preserved.**
- Future work includes long term imaging experiments in order to study cellular processes that involve morphological changes e.g. blebbing, cell division or migration.

References: [1] B. Bailey et. al., *Nature*, 1993. **[2]** P. Tinning et. al., *Biomed. Opt. Express.* 2018.