**TITLE:** Total Internal Reflection Fluorescence Microscopy with Improved Background Suppression

**INVENTOR:** Reto Fiolka

**TECHNOLOGY:** Medical Devices

**UTSD:** 3118

**SUMMARY:**

This technology describes a method to reject virtually all out-of-focus blur in Total Internal Reflection Fluorescence (TIRF) microscopy, thereby greatly increasing the clarity and signal-to-noise ratio in the final image.

TIRF microscopy is a widely used technique to selectively image fluorescent proteins adjacent to a glass cover slip. Its unrivaled sensitivity and signal-to-noise ratio have enabled the observation of endocytosis, exocytosis, protein trafficking, and virus dynamics, to name just a few examples.

TIRF uses an evanescent field that is generated by total internal reflection at a refractive index interface. The evanescent field only penetrates around 100nm into the sample, creating a very thinly illuminated optical section.

A TIRF microscope can also be operated in a different mode where the illumination laser beam is not internally reflected, but launched into the sample at a high inclination angle, also known as Highly Inclined and Laminated Optical sheet (HILO).

In practice, the TIRF evanescent field is scattered by the sample and optical imperfections, resulting in out-of-focus blur and increased background compared to the theoretical expectations. As there is no mechanism to reject such blur in a TIRF microscope, the final image will be contaminated and appear less sharp.

Because HILO allows to image deeper within the sample, even larger amounts of out-of-focus blur are inevitably generated compared to TIRF microscopy.

The invention allows suppression of out-of-focus blur without losing any in-focus light, thereby achieving a much greater sensitivity than competing technologies (such as confocal microscope), improving image clarity and signal-to-background ratio, as well as dramatically improving TIRF and HILO microscopy image analysis and interpretation.

Please contact the Office for Technology Development for more details:

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