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2 Classical optics and microscopy

2.2 Methodology

- 2.2.1 Conventional wide-field optical microscopy
- 2.2.2 Interference contrast microscopy
- 2.2.3 Phase contrast microscopy
- 2.2.4 Fluorescence microscopy
- 2.2.5 Confocal light scanning microscopy (CLSM)
- 2.2.6 Total internal reflection microscopy (TIRF)

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Widefield Microscopy: Contrast

The diagram illustrates the optical path in widefield microscopy. At the top, a layer of self-luminescent objects (red circles) is shown on the object plane. Light rays from these objects pass through an objective lens and a tube lens to be focused onto the image plane. The image plane shows a magnified and inverted image of the objects. Labels include: Self-luminescent objects, Object plane, Objective, Tube lens, and Image plane.

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Widefield Microscopy: Contrast

This diagram shows a plane below the focal plane of the objective lens. Light rays from self-luminescent objects (red circles) on the object plane pass through the objective and tube lenses. The image plane is also below the focal plane of the tube lens, resulting in a blurred image. Labels include: Self-luminescent objects, Object plane, Plane below focus, Objective, Tube lens, Image plane, and Plane below image.

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Confocal Microscopy - Principles

The diagram illustrates the principle of confocal microscopy. An excitation beam (blue arrow) is focused through an objective lens onto a plane below the focal plane of the objective. Light from the focal spot passes through the objective and tube lenses. An aperture in the image plane, known as a pinhole, is placed at the focal point of the tube lens. Light passing through the pinhole is collected by a detector. Labels include: Excitation beam, Object plane, Plane below focus, Objective, Tube lens, Aperture in image plane ("Pinhole"), and Detector.

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KIT Confocal Microscopy - Principles

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KIT TIRF-Microscopy

Total Internal Reflection Fluorescence

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Olympus

KIT TIRF-Microscopy

Figure 7

Figure 2

Nikon Plan Apo 60X/1.40 Oil DIC H 06.0.17 WD 0.21

Olympus, Nikon

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KIT TIRF: Example


Fluorescing beads in watery solution (Brownian motion)

Epi-Fluorescence

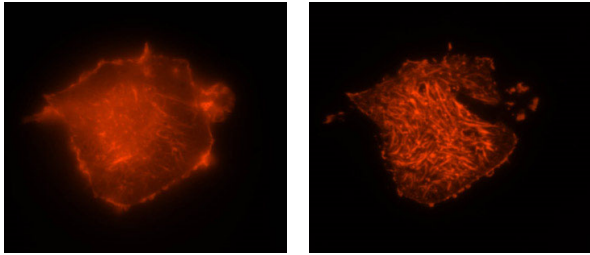
TIRF

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Zeiss

 **TIRF: Example**

Epi-Fluorescence TIRF



Phalloidin F-Actin binding protein coupled to Alexa-546 dye, actin filaments, cytoskeleton around the submembrane cortex in bovine cells.

(Dr. Klingauf, MPI für Biophysikalische Chemie, Göttingen)

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