TIRFm
(Total Internal Reflectance Fluorescence Microscopy)

New at the OCS Microscopy Core

Lunch Talk March 2015

Michael Cammer
Nikon Eclipse Ti microscope
2010: Purchased with lasers for TIRF by Dr. Michael Dustin
2014: Transferred to Microscopy Core and upgraded by OCS for epifluorescence (LED light sources, new computer, additional lenses & sCMOS camera)
Technical Details

• Nikon Eclipse Ti inverted microscope
• Environment chamber with heat unit
• Motorized stage for tiling and multiple fields imaging
• Autofocus stability
• NIS-Elements software
## Technical Details

<table>
<thead>
<tr>
<th>Lens Type</th>
<th>N.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plan Fluor 40x DIC M N2</td>
<td>0.75</td>
</tr>
<tr>
<td>Apo TIRF 100x Oil DIC N2</td>
<td>1.49</td>
</tr>
<tr>
<td>Plan Fluor 10x Ph1 DLL</td>
<td>0.30</td>
</tr>
<tr>
<td>Plan Apo 20x DIC M N2</td>
<td>0.75</td>
</tr>
<tr>
<td>S Plan Fluor ELWD 40x Ph2 ADM</td>
<td>0.60</td>
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<tr>
<td>Plan Apo λ 60x Oil Ph3 DM</td>
<td>1.40</td>
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</tbody>
</table>
## Technical Details

Standard Epifluorescence with Andor Zyla sCMOS Camera

<table>
<thead>
<tr>
<th>Excitation wavelengths</th>
<th>Emission wavelengths</th>
</tr>
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<tbody>
<tr>
<td>nm</td>
<td>nm</td>
</tr>
<tr>
<td>395</td>
<td>435/26</td>
</tr>
<tr>
<td>440</td>
<td>475/20</td>
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<tr>
<td>508</td>
<td>515/30</td>
</tr>
<tr>
<td>555</td>
<td>540/21</td>
</tr>
<tr>
<td>640</td>
<td>595/40</td>
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</tbody>
</table>

**Dichroics**

- CFP/YFP/Dsred
- Dapi/FITC/TxRed/Cy5

**Frequencies**

- 550 nm
- 575 nm
- 700 nm
- 750 nm
Technical Details

TIRF with Andor DU897 Camera

Excitation Lasers
405 nm
488 nm
561 nm
641 nm

Chroma 97327 C-TIRF
zet405/488/561/635x quad-band clean-up/excitation filter
zt405/488/561/640rpc

Filters in external emission wheel:
ET450/40M
ET525/50M
ET600/50M
ET700/75M
Before we discuss TIRF, highlights of the Nikon microscope in standard modes.
Large Tissue Scanning
Multiple Field Timelapse

- Multiple Colors
- Multiple fields
- Timelapse
Multiple Field Timelapse

• Multiple Colors
• Multiple fields
• Timelapse

Find a lot of events for statistics or find rare events
Multiple Field Timelapse

- Multiple Colors
- Multiple fields
- Timelapse

9 fields, 2 colors + transmitted, 2 min intervals for 8 hours (12 GB data)

Find rare events

Evgenia Korol in Mamta Tahiliani lab
The microfluidic platform is designed to enable perfusion based microenvironment control for long term, high quality live cell microscopy.

Continuous perfusion of culture medium to the cells recreates the physiologic mass transport condition for optimized cell health, giving a suitable growth environment for long-term experiments from 4-72 hours on the microscope stage.

The system enables single or multi-cell tracking while automated perfusion controls washout, drug changes, and dynamic solution profiles.

Temperature and CO₂ control is maintained by an on-chip microincubator.
TIRF (Total Internal Reflectance Fluorescence)

Standard Epifluorescence

TIRF

Higher contrast of molecules at substrate

http://podosome.com
50 to 200 nm Z Axis “Resolution”

• Technically, the spatial resolution in the Z axis isn’t improved.
• The energy activating the fluorescent molecules is limited to a depth of 200 nm maximum.
• Effectively, the result is imaging molecules only within 50 to 200 nm of the substrate, or effective resolution of 50 – 200 nm in the Z axis.

How do we do this?
Total Internal Reflectance Fluorescence Microscopy is based on an evanescent field that is produced at the critical angle between two interfaces of different refractive indexes.
Approaching Critical Angle
How It Works

Internal Reflection

Schematic from Duke microscopy website
Chromatic Aberration

Different colors of light focus on different focal planes. This is a problem in microscopy where you want to take pictures of violet through near infra-red fluorescent emitters. When you focus on the green fluorescence, with all microscopes to some extent the violet and far-red images are out of focus.

Problem

Solved
Practicalities

# 1.5 coverslip

Not #0, not #1...

http://microscopynotes.com/coverslips/index.html
Example #1.5 Coverslip Bottom Chambers

http://microscopynotes.com/coverslips/index.html
http://www.bioptechs.com/Products/FCS2/fcs2.html
Sequential Colors

<table>
<thead>
<tr>
<th>Time Intervals Seconds</th>
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</thead>
<tbody>
<tr>
<td>14.95</td>
</tr>
<tr>
<td>15.11</td>
</tr>
<tr>
<td>14.99</td>
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<td>14.91</td>
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<tr>
<td>14.91</td>
</tr>
<tr>
<td>15.02</td>
</tr>
<tr>
<td>15.00</td>
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To go faster, need to use fewer imaging modes and fewer colors. Easy to sequence different conditions; run fast single color then slower multiple channels.
Example Quantification: Radial Intensity Plots Show Locations of Molecules Per Cell Compartments

En face view of the synapse with cSMAC, pSMAC and dSMAC and en face view of a kinapse.

From Dustin, 2011
Primary Cilia

Primary cilium in fibroblasts (marker acetylated α tubulin)

sticking out on top:

coverslip

unpublished Linda Schneider (P. Satir & S.T. Christensen labs) & Michael Cammer - 2006
Primary Cilia

Primary cilium in fibroblasts (marker acetylated α tubulin)

The primary cilium is on the bottom of the cell!
Allows for high contrast imaging of MT or associated proteins (motors, receptors, etc.) in intact primary cilia.
From standard epifluorescence to TIRF in an f-actin in vitro assay

Rob Eddy
unpublished

Anchor points closer to substrate are brighter
TIRF FRET

Active WASp is localized in podosomes and its activity is required for podosome maintenance. WASp is active in podosomes. RAW/LR5 cells transfected with a WASp biosensor, fixed and stained with Alexa Fluor 568-phalloidin and imaged by TIRF microscopy.

Hi Lo may be useful for thick samples
Early C. elegans embryo imaged with near-TIRF illumination to overcome the problem of the 200nm-thick eggshell that makes it difficult to use the true TIRF optics. The molecule here is Par-6-GFP a polarity protein. This method allows measuring the exchange rate and mobility of the single molecules at the membrane.

Yuliya Zilberman in Nance lab
Correlative TIRF and TEM

**g.** T cell with centrally accumulated GAG-GFP resuming motility and releasing GAG-GFP-containing microvesicles. **h.** Higher magnification image of boxed region in **g.** showing internal juxta-membrane density in GAG-containing microvesicles. Arrowhead, plasma membrane.

Official Website:
http://www.med.nyu.edu/ocs/microscopy

MC’s Personal notes site:
http://microscopynotes.com/

This talk without movies at:
http://microscopynotes.com/tirftalk.pdf
(draft as of 20150330_1344)