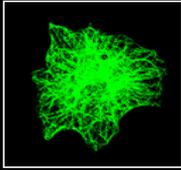


Confocal Microscopy Core of the Cell and Cancer Biology Branch

Yvona Ward and Kathleen Kelly

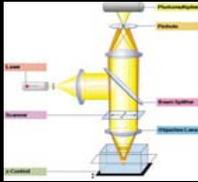
Introduction

Confocal or laser scanning microscopy is a powerful tool for visualization of 3-dimensional localization of organelles or molecules in live or fixed specimens. Confocal microscopy is distinct from conventional fluorescent microscopy because light from the specimen is collected through a pinhole conjugate to the focal plane of the specimen. Consequently, only light originating from the focal point can pass through the pinhole while any "out of focus" light is excluded resulting in a well-defined image. This unique feature makes confocal microscopy an ideal technique for 3-dimensional analysis of specimens. The Zeiss LSM 510 in the CGBB core facility has been used to address many important research questions. We have looked at translocation and colocalization of proteins in live and fixed cells, effects of interfering RNA's on development of vasculature in a Zebrafish embryo model, and formation of ascini by immortalized human breast epithelial cells. Recently we have used the ability of the confocal microscope to generate high-resolution photosessions through relatively thick specimens to investigate the potential of fibrosarcoma HT1080 cells and thyroid carcinoma KAT4 cells to migrate through Matrigel. This model will be used to address the role of a heterotrimeric G-protein coupled receptor, CD97, in invasion.



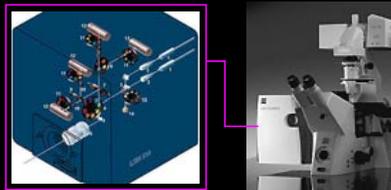
Confocal microscopy is ideal for generating high resolution images. Microtubules were stained with monoclonal antibody against α -tubulin and FITC-conjugated goat anti-mouse secondary antibody. A 3-D maximal projection is shown.

Confocal Microscopy



Simplified optics of a confocal microscope. Laser light is directed through the fiber optics cable to the specimen. The fluorophores in the specimen become excited and emit light which passes through the dichroic beam splitter and is focused into the pinhole. The light that passes through the pinhole is measured by the detector or photomultiplier tube.

LSM 510

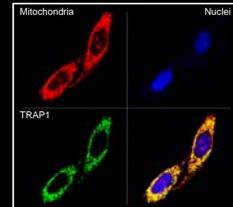
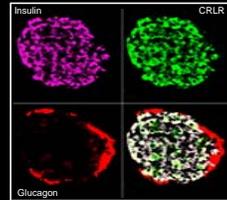


To the Specimen
 (1) optical fibers
 (4) main dichroic beam-splitter
 (5) scanner mirrors
 (6) scanning lens

From the Specimen
 (1) optical fibers
 (4) main dichroic beam-splitter
 (7,8,9) secondary dichroics
 (10) pinhole diaphragm
 (11) emission filters
 (12) photomultipliers

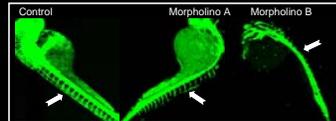
Colocalization

Three-color immunofluorescence showing complete colocalization of insulin (Cy5) with calcitonin receptor-like receptor (FITC) but not with glucagon (rhodamine) in rat pancreatic islets.

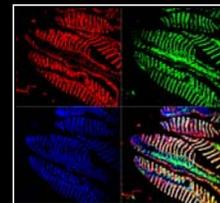


Localization of TRAP1 to mitochondria in PC3-M cells. Mitochondria were identified using MitoTracker Orange CMTMRos (Molecular Probes Inc.) and DAPI was used to visualize nuclei. Trap1 was stained with monoclonal antibody followed by a FITC-conjugated secondary antibody.

Zebrafish

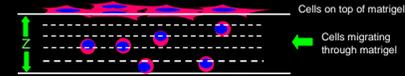


The endothelial-specific *fl/1* promoter was used to drive the expression of enhanced green fluorescence protein (EGFP) in zebrafish blood vessels (Lawson and Weinstein, 2002). This allows visualization of the vasculature in live embryos and adult fish and facilitates screening of agents and mutations that disrupt vessel development. 3-D projections of embryos 32 hours post-fertilization are shown.



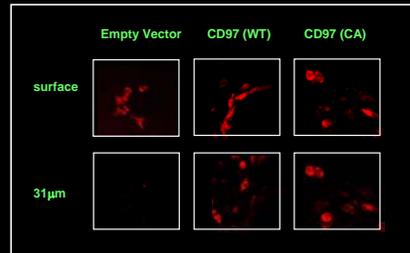
Zebrafish gills. Three different components were stained with immunofluorescent antibodies.

Cell Migration Through a 3-D Matrix



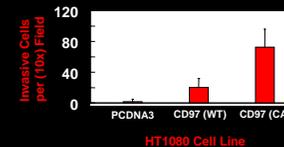
Cells are plated on a 500µm layer of matrigel with growth factors and are allowed to migrate for 24 hours. Photosessions through the matrigel are generated using confocal microscopy and cells at each depth can be quantified.

Morphology of HT1080 Fibrosarcoma Cells in 3-D Matrigel Culture



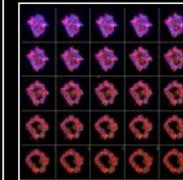
Cells were allowed to migrate into matrigel for 24 hours. Cells that migrated faster and further into the matrix, had a rounded cell morphology whereas those that were less motile had a flatter phenotype.

Migration of HT1080 Fibrosarcoma Cells in 3-D Matrigel Culture

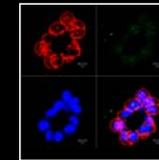


HT1080 cells transfected with wild-type (WT) or constitutively active CD97 (CA) migrate further through matrigel.

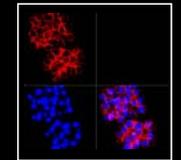
Formation of Ascini in 3-D Cultures



Three-dimensional culture of MCF10A mammary epithelial cells on a reconstituted basement membrane leads to the formation of polarized, growth arrested ascini-like spheroids that recapitulate several aspects of glandular architecture in vivo. Introduction of oncogenes into MCF10A cells disrupts this morphogenetic process and results in distinct morphological phenotypes.



MCF10A cells in 3-D culture form ascinar structures consisting of polarized cells surrounding a hollow lumen.



Transformation of MCF10A cells with RasV12 results in complex multilaminar structures that lack the hollow lumen.

Core Equipment

Inverted fluorescent microscope (Zeiss Axiovert 100M)
 LSM 510 scanning module and lasers for 4-color confocal

Inverted fluorescent microscope (Zeiss Axiovert 100M)
 Microinjection apparatus (Eppendorf Transjector 5246)
 Digital Camera (Nikon DXM1200)

Upright fluorescent microscope (Zeiss Axioplan 2)

Workstation
 Computer (Dell Precision 530) and 2 Monitors
 Printer (HP Color LaserJet 4550)

Literature Cited

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