

BioS368 Cell Biology Laboratory at Lehigh University: Cell Culture and Fluorescence Microscopy



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Cell culture and fluorescence staining/labeling techniques that allow observing organelles, proteins and nucleic acids in cells are important techniques that are applied by Cell Biologists all over the world. The Cell Biology Laboratory at Lehigh is a unique experience to learn and apply state-of-the-art fluorescence microscopy techniques. The course has four main sections: (1) Students thoroughly learn how to culture immortalized cell lines, (2) to stain sub-cellular structures in fixed and living cells using specific probes and antibodies (including double and triple color labeling), (3) to express and observe proteins tagged with fluorescent protein probes (GFP and derivatives, RFPs) in living cells, and (4) to interfere with cellular processes using specific drugs. Pursued experiments are not standard experiments available commercially in kit form, but are based on actual, unique research projects pursued in the instructor's laboratory that have been adapted to the classroom. Students maintain their own cells during the entire course and grow cells in dishes and on cover slips for experimental manipulation and microscopic examination. The course is designed to give students a hands-on experience in cell biological experimentation, and even to contribute directly to discovery! Interested? Note, that all cell images presented here were acquired by previous BioS368 Cell Bio Lab students.

Cell Culture

Cell culture under the laminar flow hood

HeLa cell growth curve and calculating duplication time

Splitting cells

Counting cells with a hemacytometer

HeLa cells seeded on different matrices

HeLa Cells in Culture they look as other non-cancerous cells do!

Phase Contrast

Differential Interference Contrast, DIC

Contaminations:

- Mold
- Rust
- Bacteria

Lots of cells in the incubator

Counting cells

Spontaneous cells/starting culture from scratch

Endoplasmic reticulum (ER) stained with ER-Tracker Blue-white in a live HeLa cell. Note the blue filamentous staining throughout the cytoplasm.

Microtubule dynamics live cells were incubated in nocodazole to disrupt microtubule polymerization. Nocodazole was then washed out and after 30 minutes cells were analyzed. All microtubules present only in short bursts. The microtubule reorganization is visible in the cell periphery.

Cell-cell junctions (Adherens, Desmosomes) required to maintain fixed protein domains. In these cells, adherens and desmosomes are stained for E-cadherin and beta-catenin, respectively. Note the localization of E-cadherin in the cell-cell junctions and beta-catenin in the cell periphery.

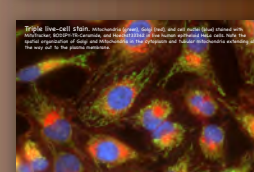
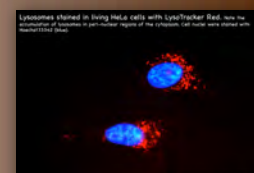
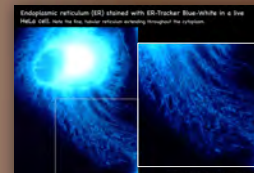
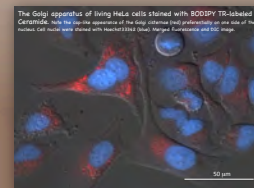
Cell-cell communication via gap junctions Chemicals entered only if neighboring cells were connected via gap junctions. Cells were stained for calcein (green) and DAPI (blue) to stain nuclei. Cells were then treated with calcein (green) and DAPI (blue) to stain nuclei. Cells were then treated with calcein (green) and DAPI (blue) to stain nuclei.

Microtubule-Microtubule Interactions microtubules (red) were stained with tubulin (red) and microtubule-associated protein (MAP2) (green). Note the localization of MAP2 in the cell periphery and tubulin in the cell periphery.

Expression of green (GFP) and red (DsRed) fluorescent proteins is necessary to visualize live cells. Cells were stained with tubulin (red) and microtubule-associated protein (MAP2) (green). Note the localization of MAP2 in the cell periphery and tubulin in the cell periphery.

Photobleaching live cells were transiently transfected with tubulin (red) and microtubule-associated protein (MAP2) (green). Cells were then treated with calcein (green) and DAPI (blue) to stain nuclei.

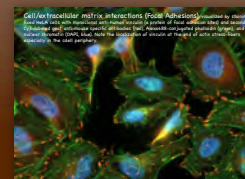
Live-Cell Stains



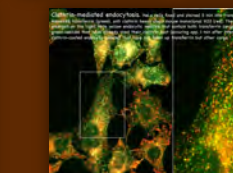
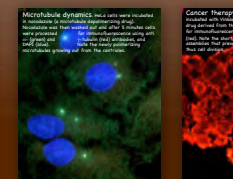
Fluorescence Microscopy



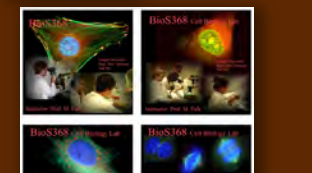
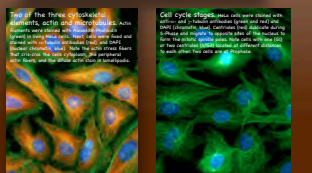
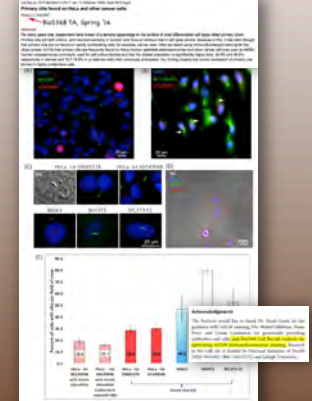
Stains in Fixed Cells



Functional Assays



Discovery



Fluorescent Proteins (GFP, RFP, Dendra2, etc.)



Acknowledgements. I thank the many generations of Lehigh students who have taken my class, showed their excitement and dedication, and had fun learning hands-on science, and contributing to discovery!