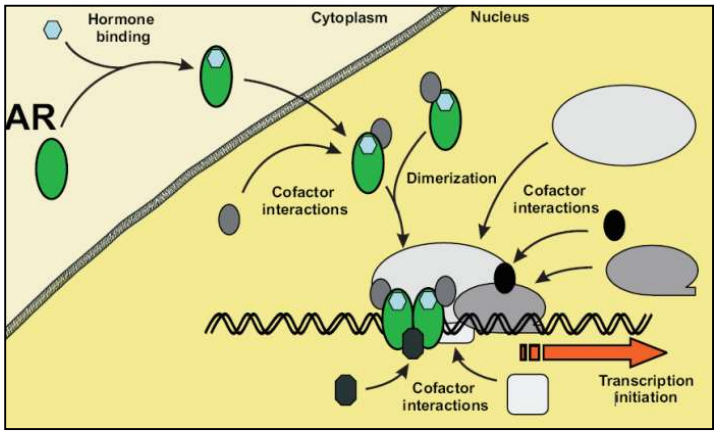
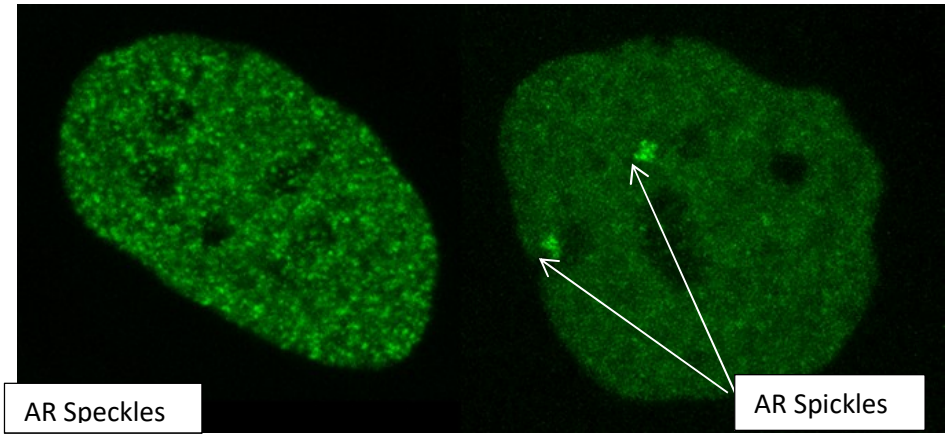
Time Lapse imaging YFP-Androgen Receptor-CFP (YARC) in Hep 3b cells on a confocal microscope

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Introduction

The androgen receptor (AR) is a ligand-dependent transcription factor. Upon activation, it binds to hormones such as testosterone or dihydrotestosterone (DHT) in the cytoplasm before translocating into the nucleus. The primary role of the androgen receptor is to act as a DNA-binding transcription factor, controlling gene expression. It also regulates genes crucial for the development and maintenance of the male sexual phenotype. This molecule, the androgen receptor, plays a vital part in coordinating genetic responses to androgenic hormones, which are integral to the development and functioning of male secondary sexual characteristics.





For this study, we will be using the Hep3b cells stably expressing the Androgen receptor gene fused to the gene encoding for the Cyan fluorescent protein (CFP) and Yellow fluorescent protein (YFP), YARC

Practicum:

The cells are already mounted and transferred to a heated (37°C) stage of the microscopic, under 5% CO2 conditions throughout image acquisition.

- In this session, we will acquire multiple time-lapse movies from different fields of views to ensure a representative samples. Find the cells expressing YARC and configure all the imaging parameters desired to acquire images, including z-stack settings, scan speed, laser power, gain, offset, frame averaging, and resolution.

Leica SP8 microscope:

- Start the LAS X software and initialize the microscope stage.
- Setup the imaging parameters (lasers, detectors, etc) and focus your sample.
- Select the Mark and Find mode and follow the protocol that it will be provide during the practicum.

Data Analysis*:*

I. Calculate the percentage of dividing cells from the generated time-lapse movies.

II. Compare the amount of AR speckles in daughter cells to that in the mother cell. Select at least two mother cells and their corresponding daughter cells. For image analysis, Macro's will be provided during the practicum. Here is what you can measure:

- **Number of Speckles:** determine the individual AR speckles in each nucleus
- **Size:** Measure the size (area) of individual nucleus.
- **Intensity:** Quantify the fluorescence intensity within each nucleus/ speckle to assess its brightness.

III*. Within the 3D cell structure, attempt to determine:

- The localization of speckles.

- The quantity of speckles.
- The duration for which the speckles are present.

*Bonus Assignment:

CONFOCAL MICROSCOPY

(from http://www.microscopyu.com/articles/confocal/confocalintrobasics.html)

Confocal microscopy offers several advantages over conventional optical microscopy, including shallow depth of field, elimination of out-of-focus glare, and the ability to collect serial optical sections from thick specimens. In the biomedical sciences, a major application of confocal microscopy involves imaging either fixed or living cells and tissues that have usually been labeled with one or more fluorescent probes.

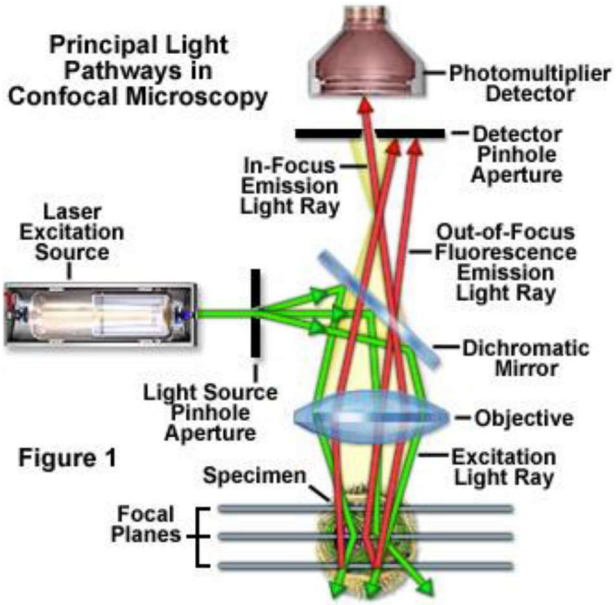


Figure 1

When fluorescent specimens are imaged using a conventional widefield optical microscope, secondary fluorescence emitted by the specimen that appears away from the region of interest often interferes with the resolution of those features that are in focus. This situation is especially problematic for specimens having a thickness greater than about 2 micrometers. The confocal imaging approach provides a marginal improvement in both axial and lateral resolution, but it is the ability of the instrument to exclude from the image the "out-of focus" blur that occurs in thick fluorescently labeled specimens, which has caused the recent explosion in popularity of the technique. Most current confocal microscopes are relatively easy to operate and have become part of the basic instrumentation of many multi-user imaging facilities. Because the resolution possible in the laser scanning confocal microscope (**LSCM**) is somewhat better than in the conventional widefield optical microscope, but still considerably less than that of the transmission electron microscope, it has in some ways bridged the gap between the two more commonly used techniques. Figure 1 illustrates the principal light pathways in a basic confocal microscope configuration.

In a conventional widefield microscope, the entire specimen is bathed in light from a mercury or xenon source, and the image can be viewed directly by eye or projected directly onto an image capture device or photographic film. In contrast, the method of image formation in a confocal microscope is fundamentally different. The illumination is achieved by scanning one or more focused beams of light, usually from a laser, across the specimen (Figure 2). The images produced by scanning the specimen in this way are called optical sections. This terminology refers to the noninvasive method by which the instrument collects images, using focused light rather than physical means to section the specimen.

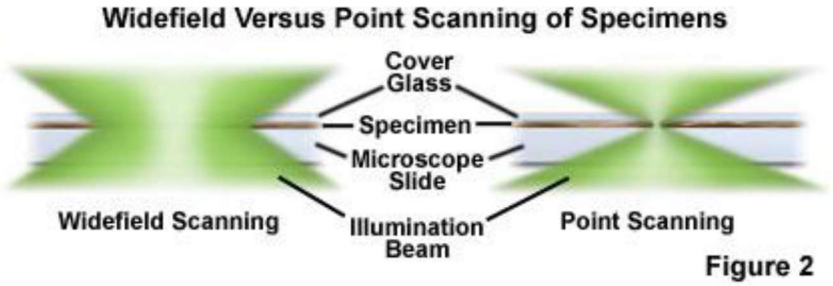


Figure 2

The confocal approach has facilitated much more useful imaging of living specimens, enabled the automated collection of three-dimensional (z-series) data, and improved the images obtained of specimens using multiple labeling. Figure 3 presents a comparison of a conventional epifluorescence image with a confocal image of similar regions of a whole mount of a butterfly pupal wing epithelium stained with propidium iodide. There is a striking improvement of resolution of nuclei in the LSCM image due to elimination of out-of-focus fluorescence flare.

The laser scanning confocal microscope (LSCM) is currently the most widely used confocal variation for biomedical research applications. Emphasis is placed on the LSCM in this introduction, since it is the design most likely to be encountered by the novice user. Other alternative designs of the instruments are favored in specific niches within the field of biological imaging. Most of the protocols for specimen preparation can be used, with minor modification, for any of the confocal instrument variants, as well as for other methodologies for producing optical sections such as deconvolution techniques and multiple-photon imaging.