# Laser Capture Microdissection

Laser Capture Microdissection, also called LCM, is a technique that allows selective isolation of cells from complex heterogeneous tissues. In this laboratory, the method is used to isolate nematode infected cells of soybean roots.

1. In the first step of this process, pathogen infected tissue is cut into small pieces. These tissue fragments are placed in a jar with fixation buffer to remove air bubbles from the sample. The tissue is incubated overnight at 4°C to allow complete fixation of the cells.
2. The next day root tissue is dehydrated by incubating the sample progressively through a series of five ethanol solutions. Each ethanol solution is added to the jar with the roots, incubated for 30 minutes, and the solution then replaced with the next one. The 4th solution has a dye mixed with the ethanol which stains the roots.
3. The next step is to wash the stained roots with a mixture of ethanol and xylene. The sample is incubated in these graded xylene:ethanol mixtures, again for about 30 minutes each at 4°C. This step prepares the tissue for fixation.
4. A tissue casting tray is placed on a hot plate and allowed to warm to 60°C. A beaker filled with melted wax is removed from an incubator and the paraffin wax is poured into the bottom of the casting tray. The dehydrated, stained root tissue is then poured into the casting tray and oriented for sectioning.
5. The root fragments are teased apart so they spread quite evenly and in a specific orientation in the wax tray. The wax is allowed to harden with the root tissue embedded in it.
6. The hardened wax is cut into small rectangles that are used for microtome tissue sectioning. From each wax rectangle, extraneous wax surrounding the root tissue is removed, the wax block loaded on a wooden block and placed in position on the microtome.
7. The microtome is a piece of equipment that allows sectioning of tissue embedded in a solid medium, in this case paraffin. Serial sections of the root tissue are made on an optical microtome with a sectioning thickness of 10 micrometers. These tissue sections, connected in long ribbons are retrieved. These are stretched out on a clean surface.
8. LCM uses special slides that are covered with a transfer film. The film will later transfer the selected cells after tissue acquisition. Prior to sectioning, LCM slides are placed on a 42°C slide warmer and are covered with RNAse-free water. Small pieces of the sliced ribbon are placed in the water, on top of a glass slide. After drying, the tissue adheres to the film. This step is important for later isolation of selected cells. Excess water is removed by incubating the slide on the slide warmer.
9. The tissue sections are then incubated in xylene in coplin jars to dissolve and remove the paraffin wax. The slides are placed on top of a paper towel and air dried. This shows a slide with dried tissue.

10. The LCM system consists of 2 parts, a Microscope and a computer for image display. It has a microscope with an ultraviolet laser beam. This ultraviolet laser will be calibrated and the power will be adjusted before capturing the cells of interest. The slides with tissue sections are placed upside down on the slide stage of the microscope. The cells of interest can be chosen on the basis of their morphology or immunohistochemical phenotype.
11. A specific software package is used to select the infected cells by moving the cursor, shown by the red line. The laser provides enough energy to cut both the transfer film and the tissue. In this case, nematode infected cells are selected.
12. By using gravity, the selected infected tissue with adherent film sections drop into the cap of a microfuge tube that is located below the tissue sample. Because of the short exposure time no detectable damage of the cells occurs. If you look carefully the selected cells can be seen in the microfuge tube that is located under the sample. These cells can be further used for DNA, RNA or protein extraction and analysis.

As seen, precautions are taken to enter and exit this containment greenhouse. However, following these protocols and working inside this specialized greenhouse, scientists can safely study pathogens that are not native to the US soil. We hope that research performed here will help farmers prepare for the day that these pathogens do enter the United States.