LASER CAPTURE MICRODISSECTION: A PUNCH BIOPSY UNDER THE MICROSCOPE

Stephen W. Carmichael,[1] Mayo Clinic

The size and precision of a tissue sample has always been a concern. As it has become possible to analyze smaller and smaller samples with molecular techniques, one must be increasingly concerned that the sample is what we think it is. So how do you dissect out a specific cell or a small group of cells? Whereas several techniques have been suggested, a group at the National Institutes of Health (NIH) have come up with an ingenious solution. Robert Bonner, Michael Emmert-Buck, Kristina Cole, Thomas Pohida, Rodrigo Chuaqui, Seth Goldstein, and Lance Liotta have published a technique they have termed Laser Capture Microdissection (LCM).[1]

With LCM, Bonner *et al.* have demonstrated that a specific cell can be identified and transferred to an appropriate container for molecular analysis. Using the polymerase chain reaction (PCR), the genetic material from a single cell can be amplified for analysis. However, data from several cells (about 20) needs to be examined so that variations in the cell cycle, *etc.*, can be averaged.

When performing LCM, the tissue section or cytology specimen is placed on a glass slide and examined under a specially-equipped microscope. An area as small as 3 μm^2, with precision approaching 1 µm, can be selected for removal from the specimen. A special polymer transfer film is in place over the specimen. A pulsed laser beam is passed through the film and the cell(s) of interest. The film is heated just enough to melt and slightly infiltrate into the specimen. Now here's the important part: as the film re-hardens, it forms a bond with the selected specimen that is stronger than the adherence between the specimen and the glass slide. The film is transferred, along with the selected specimen, to a vial for PCR and subsequent analysis. A tiny punch biopsy has been performed!

There are two special items that have been developed for LCM; the microscope and the transfer film. The microscope focuses the laser diode beam either with the microscope objectives (in a prototype "epi-irradiation" system) or with a lens incorporated into the condenser optics (in the commercially-available system). The laser beam is focused to a discrete size and precise pulsed doses are delivered to the film directly over the selected specimen. The thermoplastic polymer film contains specific infrared-absorbing dyes that cause the film to melt under the influence of near-infrared gallium arsenide laser diodes. These lasers are relatively easy and inexpensive to incorporate into a microscope. The film does not interfere with the visible spectrum, allowing identification of stained cells. This particular film was chosen to have a steep decrease in viscosity as the temperature rises, so that it can infiltrate the specimen at temperatures that do not harm the specimen. Also, the thermoplastic bonding does not interfere with subsequent molecular analysis.

The group at NIH have entered into a collaborative arrangement with Arcturus Engineering (Mountain View, California) to provide a commercial instrument. In addition to the microscope and the film, this instrument includes a mechanism for transferring the tiny specimens for analysis, and imaging and data recording systems. Over 20 systems have been delivered. As the list of human genes specific for diseases expands, the use for such a system in a diagnostic laboratory will correspondingly expand.

Bonner *et al.* point out several potential uses for LCM, and I am sure that the list will grow rapidly. They have provided us with a rapid, reliable method for taking punch biopsies at the microscopic level! ■

1 The author gratefully acknowledges Robert F. Bonner for reviewing this article.

2. Bonner, R.F., M. Emmert-Buck, K. Cole, T. Pohida, R. Chuaqui, S. Goldstein, and L.A. Liotta, Laser Capture Microdissection: Molecular analysis of tissue, *Science* ***278***: 1481-1483, 1997

Front Page Image

High Resolution Transmission Electron Micrograph of a Lead Precipitate on a Symmetrical High Angle Tilt Grain Boundary in Aluminum

The alloy was thermally annealed to allow precipitates to develop their equilibrium shapes. Unlike the small particle seen in the upper grain which has a cubo-octahedral equilibrium shape, the grain boundary precipitate has a compound shape made of a faceted segment on the top and a rounded segment on the bottom. The apparently different crystal orientation of the two segments is due to moiré interference fringes of a single crystal lead precipitate with two different aluminum grains. The compound shape can be understood as the intersection of a cubo-octahedron with a sphere. A more detailed description of the behavior of the grain boundary precipitate shapes can be found in a paper by Johnson *et al.* in *Interface Science, 3, 279 (1996)* and in a short article by Dahmen et *al.* in the August issue of the *MRS Bulletin.* (Micrograph by U. Dahmen on JEOL ARM 1000 at the National Center for Electron Microscopy).

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Don Grimes, Editor

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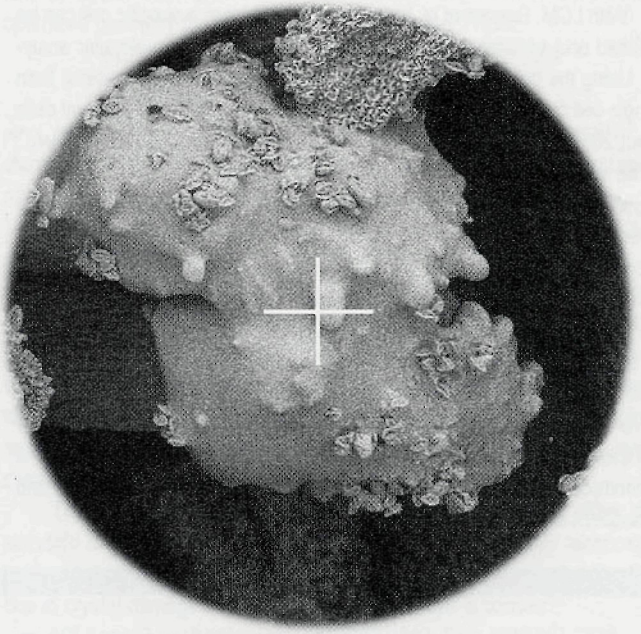

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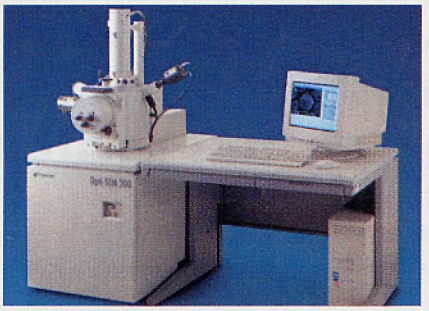

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