1. INTRODUCTION

Advances in our understanding of disease mechanisms have resulted in the need for single-cell analysis. Analytical technologies have become available to accommodate such interrogations. Typically, molecular diagnostic assays begin with a nucleic acid extraction procedure during which tissue architecture and cellular morphology is lost. Laser capture microdissection (LCM) is a technology that enables scientists to examine the processes of individual cells. Whether one is investigating a cell’s internal messages or its proteins, isolating that particular cell(s) from a mixed cellular environment is the function of LCM (Fig. 1). This chapter briefly describes the LCM technique by reviewing the current instrumentation and answers some of the most frequently asked questions about LCM.

There is now a vast literature on LCM, which this chapter will not attempt to review. A well-organized listing of the primary papers as well as contemporary work can be found at the Arcturus website http://www.arctur.com. Conn is editor of perhaps the best compilation to date on LCM (1). There is a new methods book on LCM, edited by Murray and Curran (2). LCM was introduced by the National Institutes of Health investigators Liotta, Bonner, and Emmert-Buck in 1996 (3) and 1997 (4). The first commercial instrument was produced by Arcturus Engineering, Inc. (Mountain View, CA) as a result of a Cooperative Research and Development Agreement with NIH. To date, there are four companies that produce LCM equipment.

2. LCM METHODS AND INSTRUMENTATION

Three methods exist that use lasers to collect tiny samples from heterogeneous biological specimens. The first method melts cell-sized spots of a thermoplastic film onto the specimen using an infrared (IR) laser. The plastic cools adhering to the specimen. When the film is lifted, the adhering specimen is also removed. This is the original LCM method created at NIH and first reported by Liotta et al. (3,4). This method is marketed by Arcturus (http://www.arctur.com). The second method uses a special supporting membrane under the specimen. A pulsed ultraviolet (UV) laser cuts the membrane around the desired specimen that then either drops into a collection cap by gravity (Leica; http://www.leica-microsystems.com) or is catapulted into a collection cap by a defocused laser beam (P.A.L.M., http://www.palm-mikrolaser.com). The third method uses an IR laser and a special IR-absorbing plastic film on which the sample is placed. The laser severs the film by heat, thus isolating islands of specimen which remain when the bulk of the film is removed (Bio-Rad; http://www.bio-rad.com).

2.1. ARCTURUS  The Arcturus Pix-Cell IIe is shown in Fig. 2. An automated version called the AutoPix is also available. The Arcturus LCM process is illustrated in Fig. 3. The specimen is placed on a glass slide with no cover slip. Critical to this process is the LCM cap. The cap, which is sized to fit into a 0.5-mL Eppendorf tube, is made of an optical-grade plastic with a thermoplastic film on the narrow end. The film is placed on the specimen, which is visualized using an inverted microscope equipped with a color charge-coupled device (CCD) camera and video monitor. The specimen is maneuvered utilizing the joy-stick stage positioner to place an area of interest under a target beam that appears on the monitor. The IR laser is activated by pressing a button. The laser passes through the cap from above and causes the thermoplastic film to soften and expand down into the tissue at the position of the target beam. The film adheres to the tissue. Each press of the button is called a “shot.” One cap can contain approx 6000 shots. After all areas to be microdissected have been shot, the cap with adherent specimen is lifted away from the slide. The cap is then placed into a 0.5-mL Eppendorf tube for processing of the specimen.

2.2. LEICA AS LMD  Figure 4 illustrates the Leica AS LMD. This system is based on the ability of a pulsed UV laser to cut a PEN (polyethylene–naphthalate) membrane covering a glass slide with the specimen on top. The slide is placed on the computer-controlled stage of the upright microscope, specimen side down. Fig. 5 illustrates the stage area. Areas of interest are visualized utilizing the computer-controlled stage and focus system and a color CCD camera. Specimen regions to be microdissected are outlined on the video image using the mouse cursor. Once this is done, the computer system automatically moves the stage to each location. The system guides the UV laser cutting by deflecting the laser beam through the
2.3. P.A.L.M. The P.A.L.M. system is illustrated in Fig. 6. The P.A.L.M. system also utilizes a pulsed UV laser for cutting. However, the specimen can be on a variety of substrates, including plain glass slides, slides with a PEN membrane, or culture dishes with a PEN membrane insert. The specimen is placed on the inverted microscope, specimen side up. A