Introduction
This Application Note describes a precise, rapid and convenient Laser Capture Microdissection (LCM) method for positive selection of living adherent cells and successful subsequent re-cultivation of homogenous subpopulations.

Background
Laser Capture Microdissection (LCM; Arcturus)\(^1,2\) is performed by using a near-infrared laser. In traditional applications, this laser activates a thin layer of thermoplastic transfer film which bonds specifically to cells identified and targeted by microscopy within the tissue section. The transfer film with the bonded cells is then lifted off the thin tissue section, leaving all unwanted cells behind. In this manner, a pure cell population can be obtained and subsequently analyzed.

In this Application Note, we describe an adaptation of this technique for the positive selection of living adherent cells.

**LCM for positive selection of living in vitro cells**

Adherent cells are grown in a PEN-membrane frame slide (Figure 1). Prior to microdissection, most of the growth medium is removed, and then the slide is inverted, inserted into an LCM system and imaged. Standard CapSure\(^\text{®}\) Macro LCM Caps are used as the collection devise.

Desired cells are separated from adjacent cells by pulsing the infrared laser along a complete curve surrounding the cells of interest. This activates the LCM transfer film on the CapSure caps and fuses it to the polymer membrane.

Isolation and separation of the selected cells from the cell culture occur when the cap is removed from the PEN-membrane frame slide. The bond between the fused sections of polymer film and the cap is so strong that the membrane rips surrounding these sections. In this manner, the fused sections of membrane and associated cells are transported to the cap. Undesired cells remain on the membrane frame slide. These can be captured on a second cap in a similar manner. The selected cells can then be processed for future culturing.

**Experiment: Human Breast Cancer Cells (SKBR3 cell line)**

**Materials:**
- PEN membrane frame slide (Arcturus Catalog # LCM 0521)
- Capsure Macro LCM caps (Arcturus Catalog # LCM 0201, 0202, 0211, or 0212)
- PixCell\(^\text{®}\) or AutoPix\(^\text{™}\) LCM Instrument
- Trypsin-EDTA (0.25% Trypsin, 1mM EDTA)
- Hanks’ Balanced Saline Solution
- 37°C incubator with 5% CO\(_2\)
- Pipettor and sterile pipette tips
- Sterile 96-well flat bottomed multiwell plate with cover, or cell culture flask
- SKBR3 cell culture medium:
  - 89% McCoy’s modified 5A medium
  - 9% Fetal Bovine Serum
  - 1% Sodium Pyruvate
  - 1% Penicillin / Streptomycin

**Live Cell LCM Process**

2. Invert slide and place cap over cells of interest.
3. Pulse laser on closed path surrounding the cells of interest. Repeat for additional regions of interest.
4. Remove cap with selected cells fused to the membrane.
5. Trypsinize and transfer to culture flask.

Figure 1: Laser Capture Microdissection of Living in vitro Cells.
Procedure

1. SKBR3 cells were seeded onto a membrane frame slide with 1 mL of medium, and then incubated at 37°C for four days. Fresh medium was added as needed.

2. The medium was aspirated and the back of the membrane slide was wiped with 100% ethanol to remove any moisture.

3. LCM was performed as described above using PixCell Ile and AutoPix laser microdissection systems.

4. The CapSure cap was removed from the slide, retaining the selected live cells on the transfer film.

5. 10 μl of Hanks’ Balanced Saline solution was pipetted onto the cells and incubated for 10 min at room temperature, then removed.

6. 10 μl of Trypsin-EDTA was pipetted directly onto the captured cells on the cap and incubated on the cap for 2 min at room temperature.

7. Using a 1000 μl pipette tip the Trypsin / cells were transferred into the well of a sterile 96-well, flat-bottomed plate with 50 μl of SKBR3 medium.

8. The plate was placed in the 37°C incubator.

Results:

Within 12 hours, cells attached to the well bottom. Within 24 hours, cell division could be observed. (Figure 2) Similar results were achieved using an AutoPix LCM system. (Figure 3)

Depending on cell type, the 10 min HBSS recovery described in Step 5 above may be omitted.

Discussion

Viability of this new LCM technique has been shown for normal mouse testis cells (TM3 cell line) in addition to the human breast cancer (SKBR3) cells described above. Additional techniques and protocols are under investigation. For more information, contact Arcturus at techsupport@arctur.com.

References

3. For other selected publications, go to http://www.arctur.com/research_portal/resources/index.htm.