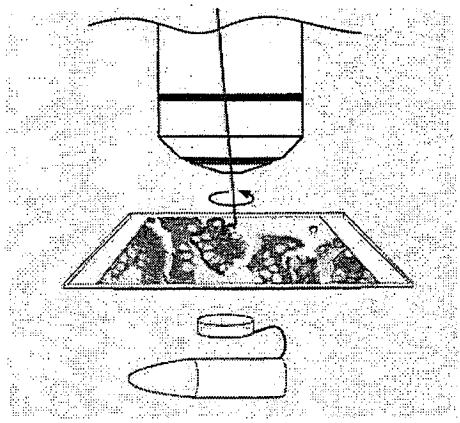
Description of Requested Instrument

- [redacted] a fully automated upright research microscope – [redacted] uses gravity as the means of capturing the dissectate. The laser microdissection system must be capable of dropping dissected material directly into a capture cap containing reaction buffer. Capture cap must be easily chosen from any one of 4, 8, 16 or 48 arrays.

- Small areas – even single cells – must be cut out with a pulsed UV laser whose specimen, particularly as the specimen is not heated. Mechanical contact is avoided to eliminate the risk of contamination. Mounting the histological section on a thin plastic film improves the cutting results and facilitates transfer to the PCR test tube. The plastic film is an innate material just like the PCR tube material.
- The Laser MicroDissection system must be based on an easy to operate turnkey system consisting of an integrated system of an upright fully automated research grade microscope, and a special two folds X-Y motorized stage for the movement of the specimen with ±10 micron precision and the collection devices beneath; remote control for all automated microscope functions and X-Y stage, Class 1 laser safety rating for the complete system, computer controlled laser control module, monochrome and/or color high resolution camera for image capture, computer, and application software that is capable of outlining an area to be micro dissected of any size and any shape from single cells to hundreds or thousands of cells on a computer monitor with a mouse or with optional pen on drawing monitor, and having the cut area fall directly into any one pre-designated of 4, 8, 16 or 48 collection tubes, containing if needed a reaction buffer. The application software must be capable of applying laser exposure to only the actual cut regions of the tissues.
- The application software must be capable of allowing multiple cuts of size and shape using objectives with magnifications of 5x, 6.3x, 10x, 20x, 40x, 63x 100x and 150x to fall directly into a designated position within a robotic tray holding up to four, eight or sixteen interchangeable PCR micro-centrifuge holders or 8-well or 48-well strips. Additionally, the software must be capable of the direct control of a motorized microscope stand and

motorized stage functions for the direct inspection of the cut tissue or cells in any one of the four or eight positions of the robotic tray.

- The laser micro dissection microscope stand must be capable of computer control of a crystal diode 355 nm laser beam steering system within the microscope imaging field of view
- Laser MicroDissection microscope stand must be capable of remote control of a motorized 7 place nosepiece, motorized Z-focus drive, motorized precision X-Y motorized stage, motorized specimen collection system, motorized condenser aperture, motorized change to Brightfield, Phase Contrast, Differential Interference Contrast (DIC). Polarized Light, or Fluorescence with the touch of one button. Koehler Illumination automatically maintained for each objective. DIC automatically set for each objective, including the contrast bias by the touch of a button. DIC prisms, polarizer and analyzer are automatically removed from the optical light path when not in DIC mode and automatically restored when DIC is chosen.



Laser Movement by optics

- The laser must be automatically focused in the visible light plane and must automatically adjust the UV offset for each objective magnification change. The laser micro dissection system must have a movable beam path so as not having the laser cutting precision limited by the stepper resolution of a motorized stage.
- Laser micro dissection microscope system must be capable of computer control of the laser module for laser apertures, laser attenuation, laser cutting speed, and UV laser focus offset, and complete laser setup/alignment via a simple dialog box.
- Laser control must have UV laser focus offset automatically adjust the laser focus to be in the same plane as the visible light focus, and to be automatically adjusted individually and separately for each objective.
- Laser calibration and alignment for each objective must be able to be performed by end-user by simple software dialog box by clicking on alignment crosses.

Laser MicroDissection must be capable of

- Saving user profiles by file name for recalling all laser module parameters (aperture, attenuation, speed, UV focus offset, and alignment) specific to each objective.
- To save and restore during actual use, the last microscope and objective parameters such as the focus position, focus sensitivity, light intensity, and condenser aperture setting as each objective is rotated into place
- Laser micro dissection with up to 7 different objectives mounted on the motorized microscope nosepiece turret.
- Utilizing objectives designed specifically for laser micro dissection (5X Microdissection, 6.3x Microdissection, L40x CORR XT, L63x CORR XT & 150x LMD objectives) with the optimal UV transmission and having correction collars (CORR) to correct for varying coverglass thickness for the highest optical performance.
- Accepting an optional yet fully integrated software allowing laser micro dissection by manual control and auto vision control laser micro dissection cutting (AVC+ or AVC+ Professional) based on color and grey level selection with subsequent feature or morphometric limitations. This software must be fully integrated and retrofittable into the basic operating software.

- Selecting a "Move + Cut" mode where the laser cutting will be activated by simply holding down the left mouse button. In this mode the laser will only continue to fire as long as the left mouse button is depressed. Optionally the "Move + Cut" mode must be capable of being activated by a touch pen and optional pen touch screen monitor.
- Designating multiple cutting areas within a given field and allowing the system to cut designated areas sequentially. Laser micro dissection microscope system must be capable of multiple cutting areas over multiple fields.
- Creating and saving an overview image of the entire microscope slide area, or over three slides. The Montage overview image must be capable of being used as a convenient navigation tool to move the motorized stage to any area of the entire microscope slide, and to digitally magnify the overview image, and to command the microscope to move to the specified objective for optical magnification.
- For a given field of designating multiple cutting areas and designating up to four different PCR tube holder or eight or sixteen well strip array positions for each designated cutting area.
- For multiple fields with 10x objective of designating multiple cutting areas and designating up to four different PCR tube holder or eight well strip array positions for each designated cutting area.
- Capturing live adherent cells grown on foil coated slides (PEN, PET or POL) for subsequent re-culture or analysis, using gentle gravity capture. Laser capture of live cells, do not radiate the live cells with laser light.
- An optional module for live cell cutting (LCC) for the isolation, capture and re-culturing of adherent cells grown in special petri dishes. This module allows laser micro dissection and direct capture of specific cells growing on special petri dishes into an eight well strip array, or a 18 well stacked cell chamber for re-culture or alternate capture into one of 4 PCR tube caps for analysis.
- An optional image database software system that automatically captures sequential images of before and after laser cutting as well as an automated image capture of the PCR tube cap inspection images.
- Not be limited to the number of objectives used for laser micro dissection and allow for automatic or user specified UV offset correction as each objective is rotated into the beam path.
- Laser micro dissection and the simultaneous live observation of the following optical techniques: brightfield, phase contrast, DIC Nomarski, and fluorescence.
- Be of modular design for field installation so as to be capable of accepting epi-fluorescence equipment holding 5 or up to 8 different fluorescence filter sets that can easily and quickly removed (less than 30 seconds) or re-sequenced without the use of tools or disassembly of the microscope system. Fluorescence axis must have fast "internal filter wheel" (IFW) for rapid exchange of up to three excitation filters for single channel or multiple channel fluorescence imaging.
- Have a Fluorescence Intensity Manager (FIM) capable of adjusting fluorescence intensity for each filter cube position, and automatically recalling last state when each fluorescence filter cube is rotated into the fluorescence illumination axis.
- Be capable of the laser micro dissection of fluorescent samples under real time observation or from a "frozen" image. When drawing the dissection cut lines on the frozen image, the excitation light is turned off, preventing photo-bleaching, quenching, or photo toxicity of the cells.
- Be capable of automated sequencing of a shuttered fluorescence illuminator and control of the transmitted illumination.
- Have a of laser safety rating Class 1.
- Have safety shield to protect specimen work area and to protect user.

[redacted] **Fully Automated Research Grade Microscope Stand:**

- Microscope must be capable for use as normal brightfield /phase contrast/DIC-Nomarski/fluorescence microscope independent of laser micro dissection system.
- Microscope must be capable of
  - motorized 7-place nosepiece.
  - motorized Z-focus with Z-step size 15 nanometers..
  - using a fully automated condenser for all contrast techniques.
  - accepting a motorized X-Y Stage with additional motorized X-Y Stage for collection tube selection.
  - accepting a single remote control device that controls the microscope nosepiece, X-Y motorized stage, condenser aperture, adjustment of the microscope lamp intensity, or any other microscope functions via four freely programmable buttons on the remote control device.
  - accepting a single remote X-Y-Z controller device with "proportionalized" capabilities that automatically adjusts for more or less X-Y-Z travel range as different objective magnifications are engaged.
  - accepting a manual X-Y stage with low coaxial X-Y adjustment controls
  - accepting a full range of interchangeable condenser systems.
  - accepting a specific condenser system for full Koehler illumination for objectives 1.25x-150x with a 7-place condenser turret system for selected compensators placement, phase and darkfield annuli, and DIC prisms.
  - using a fully automated and motorized DIC system, including a four position enclosed turret for DIC objective prisms and adjustment of DIC contrast (bias). The last used contrast (bias) must be kept in memory and restored for each objective when that objective is turned into the optical path. DIC prisms must be automatically removed from the light path and protected in a dust free environment when not in DIC mode, and capable of entering DIC mode automatically for each objective with polarizer, analyzer and proper DIC prisms in place at the touch of a button.
  - a DIC optical system that utilizes a single objective prism for objective magnifications from 5-150x.
  - simultaneous observation of DIC-Nomarski and epi-fluorescence operations.
  - accepting a POL observation tube with beam splitter with 100/0; 50/50; 0/100% settings for photographic setting.
  - POL observation tube must be capable of accepting an additional dual port beam splitter for the photographic port with 100/0; 0/100% settings.
  - accepting an optional magnification changer with built-in Bertrand lens system and magnification factors 1.0x, 1.5x, 2.0x.
  - accepting observer tubes with upright and erect image or ergo tilting tubes with photo port.
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Laser Capture Micro dissection System (LCM) instruments

- Since the human genome project offers researchers a lot of basic information about the sequences of many genes, they want to prove now the functions, the expression and the regulation of gene activity. The pure knowledge about the sequence of a gene (e.g.: atgggttacagctactgagttaaggccacgtgagat...) has to be proven for its relationship to processes within the cell (cell cycle, development, growth, diseases etc.). To do this, DNA sequences, the messenger RNA and proteins are the targets of the investigation. DNA and RNA are often further investigated by the tool of the Polymerase chain reaction, respectively RT-PCR, Southern- and Northern blotting. Protein analysis is done e.g. by SDS-PAGE, two-dimensional gel electrophoresis and Western blot analysis.

- The laser micro dissection offers researchers a tool for precise non-contact and contamination-free preparation of cell groups or single cells out of histological tissue. LCM is a technique originally devised for isolating highly selective populations of cells from a heterogeneous tissue section, cytological preparation, live cell culture and small organisms (such as C. elegans) via direct visualization of the specimens.
- In general, the LCM systems can be classified into two main categories: infrared (IR) capture systems and ultraviolet (UV) cutting systems. The principal steps involved in laser micro dissection technology are (i) visualization of the cells of interest via microscopy, (ii) transfer of laser technology to a foil polymer with formation a polymer-cell composite (IR system) or ablation of the polymer surrounding a targeted area in the specimen (UV system), and (iii) removal of the cells of interest from the heterogeneous tissue section. LCM is compatible with a variety of tissue types, cellular staining methods and tissue preservation protocols.

Experimental justification for the [redacted] Laser Micro Dissection System:

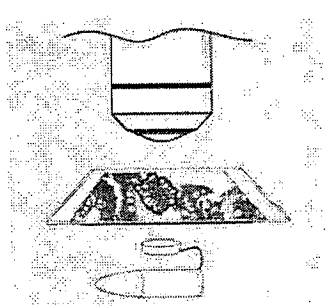
- Our requirements demand the use of a UV laser. Small areas – even single cells – are cut out with a pulsed UV laser whose focused beam is directed along the contours of the area of interest. This technology (laser ablation) guarantees extremely gentle treatment of the specimen, particularly as the specimen is not heated. Mechanical contact is avoided to eliminate the risk of contamination. Mounting the histological section on a thin plastic film improves the cutting results and facilitates transfer to the PCR test tube.
- Also of importance is that none of the other systems on the market are suitable for our application which requires an upright microscope and a non-contact contamination free gravity based collection system for the dissectates. In addition, our proposal requires that the laser micro dissection system also be capable of performing as a routine research microscope tool when not being used for laser capture micro dissection.

Most important:

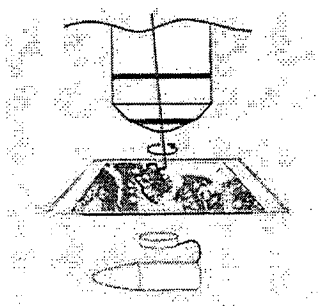
- Transport of dissectates simply by gravity – Gently and Contamination free
- Laser movement by optics (not mechanics)
- Cutting edge laser technology (adjustable and flexible)

Additional features:

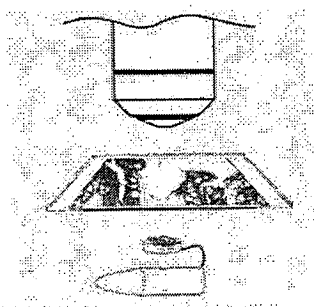
- Maximal flexibility (collection devices, consumables)
- Special optics for laser micro dissection
- Simultaneous cutting within fluorescence
- Automated recognition of cells



1. Define a region of interest



2. Laser beam steered by optics along the cutting line



3.Specimen collection by gravity