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Advancing forensics with precise target excision: the CellCut Plus laser microdissection instrument

Laser microdissection instruments, such as the CellCut Plus® and SmartCut Plus® from Olympus, have proven to be excellent research and forensic tools. This is because they provide precise extraction of biological targets such as cells and cell components from standard slide preparations, dried samples and even cell cultures, making them perfect for even the most difficult isolations. In combination with new fluorescent markers, the CellCut Plus and SmartCut Plus become even more powerful and can yield forensic data that can lead to prosecutions that were not previously possible.

Genetic fingerprinting was developed by Alec Jeffries at the University of Leicester in 1984 and has since become an essential tool in many areas, but none more so than the fight against crime. Recent advances have made it possible to apply the technique to even just a few cells. In some situations, though, obtaining a clean sample from the plethora of suspect and victim cells has proven a barrier to forensic detection. Laser microdissection (LMD), originally designed as a research tool, is now being used to overcome this barrier, providing a simple, safe and contamination-free method of extracting target cells for downstream analysis from virtually any sample, including dried cells and culture plates. Furthermore, new fluorescent cell identification processes ensure that male and female cells can be easily distinguished and extracted using LMD instruments such as the Olympus CellCut Plus and SmartCut Plus.

Tell-tail cells

Sperm cells are probably the easiest cells to distinguish purely by their shape, yet to extract them from a sample of mixed cells has generally involved several differential lysis and clearing steps. This procedure requires a favorable ratio of sperm cells to 'other' cells, which is not always the situation, and therefore some criminal cases have lacked the DNA evidence that could have led to conviction.

The introduction of LMD to the forensic scientists' toolbox has closed this gap by providing pinpoint excision of target materials using a high-power, solid-state UV laser at 355 nm (**Fig. 1**). The picosecond pulses and precise optics produce a beam spot size of less than 1 µm, providing exceptional cutting accuracy. This has enabled direct extraction of sperm cells, ensuring that even low numbers of these cells or low ratios of these cells are no longer an impediment[1]. It has also meant that previously unsolvable cases have been revisited, and convictions have been secured.

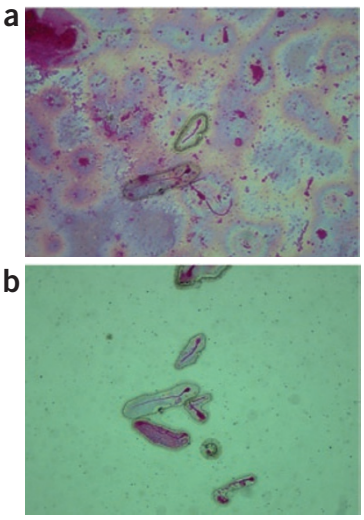

Figure 1 | Cells from a vaginal smear. (**a**) Sperm cells excised precisely using CellCut Plus. (**b**) Sperm cells collected cleanly using the unique CapLift system.

Laser microdissection

Laser microdissection systems, such as the Olympus CellCut Plus (**Fig. 2**), are highly flexible, as they are based on research-quality microscopes, and can therefore be used to excise cells from dried samples on slides or cells in liquid culture. Furthermore, cells can be identified using standard brightfield contrast staining such as Gill's Hämalaun, hematoxylin and eosin and antibodies to sperm, or fluorescent labels such as acridine orange. An unlimited number of targets can be

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Figure 2 | Olympus CellCut Plus with PenTouch monitor.

Figure 3 | MultiCap CapLift system.

identified by the user for microdissection, either by drawing around them freehand using the PenScreen tool or by selecting from predetermined shapes such as a circle or a square. For enhanced consistency, the freehand or predetermined cutting guides can be copied and pasted to ensure that each extracted target has the same excision area. The software then takes over and automatically excises all the identified targets using the laser.

Central to the isolation concept of the CellCut Plus is the contact-free recovery of the dissected targets using the fully automated CapLift system. This method, together with new software options, allows multiple samples to be collected into the same microtube cap in a predetermined layout, where they can be inspected and ablated if necessary after microdissection. The system can be expanded with the MultiCap module (**Fig. 3**) to automatically collect samples from up to three slides into eight isolation caps. The contamination-free nature and multiple target capabilities of LMD may also make it an instrument of choice for a growing number of other forensic isolations.

Distinguishing men from women

Another gap in molecular forensics has also recently been closed with the introduction of protocols for the gender-specific isolation of diploid cells, which cannot be distinguished by conventional staining methods. This may, for example, be of interest in cases with aspermatozoic or vasectomized perpetrators, or with generally unfavorable mixtures of all kinds of biological material from male and female individuals.

Initial investigations into sex-specific differentiation focused on digoxigenin-labeled Y chromosome probes. This method is reasonably simple and involves hybridization of the labeled probe followed by detection with a secondary enzyme-linked antibody and a corresponding chromogenic substrate. This stains male cells purple, as they are the only ones containing Y chromosomes. In combination with LMD, this method allows isolation of single male cells from a mixture with many female cells.

Conversely, however, isolation of single female cells from mixtures containing many male cells may also be relevant, and the digoxigenin method has a high false-positive rate. Therefore, to obtain different fluorescent signals for both male and female cells, the X/Y-probe kit from Vysis may be used. A Spectrum Green–labeled Y chromosome–specific probe hybridizes to the Y chromosome, and a second, Spectrum Orange–labeled probe hybridizes to the X chromosome. As a result, female cells show two red signals, whereas male cells are recognized by one green and one red signal. Again, LMD can be used to quickly and cleanly isolate the desired target cells for downstream processing. (For further information see ref. 2.)

Other uses of LMD

Hair samples are important in forensic investigations and can hold more clues than previously thought. Microscopic examination of hair structure including thickness measurements and cross-sections are important, and contact-free cutting of the hair can provide access to the few, mostly telogenic, hairs that contain severely degraded but still usable DNA.

Many samples from crime scenes are recovered using specimen mounting tapes, and LMD can be used to isolate and collect target material directly from these tapes, making LMD an essential tool for all modern forensic laboratories.

Conclusions

Forensic investigations are becoming more and more indomitable with the introduction of new instruments and protocols. In many situations where molecular evidence exists but can not be isolated using previously existing techniques, laser microdissection can provide the target material in a clean and usable format. The proof of the power of the new tool has been the conviction of criminals from 'cold cases' in which samples have been reexamined and target material has been successfully recovered for DNA analysis.

ACKNOWLEDGMENTS

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