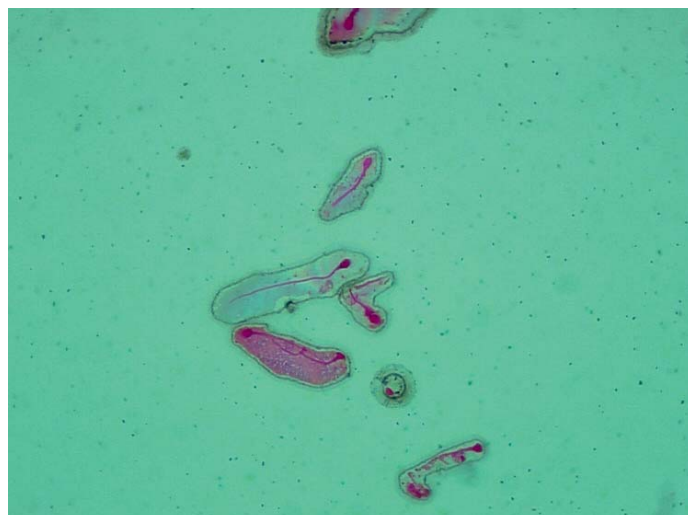
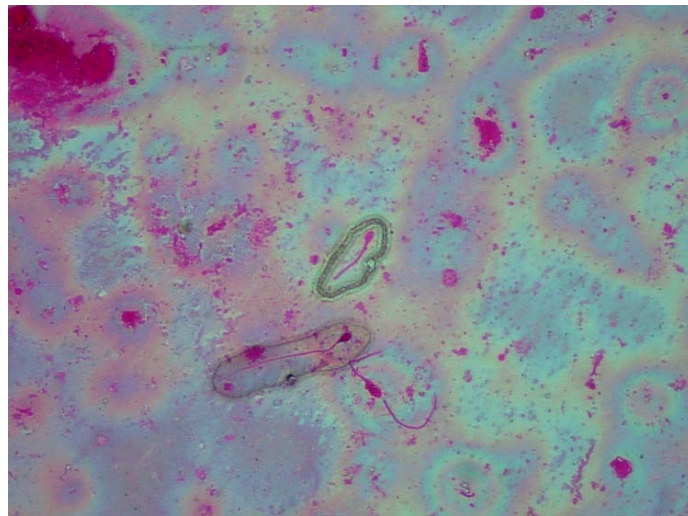


Forensics at the cellular level

The role of laser microdissection

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Genetic fingerprinting was developed by Professor Alec Jeffries at the University of Leicester in 1984 and has 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 the cellular level. In some situations though, obtaining a clean sample from the plethora of suspect and victim cells has proved a barrier to forensic detection. Laser microdissection (LMD) is now being used to overcome this barrier, providing a simple, safe and contamination free method of extracting the target cells for downstream analysis. Furthermore, new fluorescent cell identification processes ensure that male and female cells are easily distinguished and extracted using LMD.



Cells from vaginal smear. Above: Sperm cells excised precisely using CellCut Plus. Below: Sperm cells collected cleanly using the unique CapLift System.

Tell-tail cells!

Sperm cells are probably the easiest cells to distinguish purely from their shape and yet to extract them from a sample of mixed cells has generally required a number of differential lysis and clearing steps. This does mean though, that it is important to have a favourable ratio of sperm cells to 'other' cells, which is not always the situation and therefore a number of cases have lacked the DNA evidence that could have led to conviction.

2 Olympus CellCut Plus with PenTouch monitor



The introduction of LMD into the forensic scientists' toolbox has closed this gap by providing pinpoint excision of target materials via the use of a high power, solid-state UV laser at 355nm. The picosecond pulses and precise optics produce a beam spot size of less than 1µm providing exceptional cutting accuracy. This has enabled the direct extraction of sperm cells, ensuring that even low numbers or poor ratios are no longer an issue. Katja Anslinger's group and others have shown that only 30 collected sperm cells are enough to generate a full genetic profile. It has also meant that previously unsolvable cases have been revisited and convictions secured.

Laser microdissection

Laser microdissection systems, such as the Olympus CellCut Plus, do not limit flexibility either, since they are based on research level 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 bright-field contrast staining such as Gill's Hämalaun, Haematoxylin Eosin (HE) and anti-sperm antibodies, or via fluorescent labels such as acridine orange. To label the cells of interest for microdissection the user can either draw freehand around an unlimited number or use predefined shapes. The software then takes over and automatically excises all the identified targets using the laser. Central to the isolation concept on 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 enables multiple samples to be collected onto the same micro-tube cap in a pre-determined layout, where they can be inspected and ablated if necessary after microdissection. The system can be expanded to automatically collect samples from up to 3 slides into 8 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 normal staining methods. This may, for example, be of interest in cases with aspermatozoic or vasectomised perpetrators, or with generally unfavourable mixtures of all kinds of biological material from male and female individuals.

3 MultiCap CapLift system



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-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.¹

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. Laser microdissection for instance, has shown that in many situations where molecular evidence exists but cannot be isolated using existing techniques, it can provide the target material in a clean and useable format. The proof of the power of the new tool has been the conviction of criminals from 'cold cases' where samples have been re-examined and target material successfully recovered for DNA analysis.

ACKNOWLEDGEMENTS

References

1. Anslinger et al., J I Leg Med (2007)

Figures

- 1 Isolation of sperm (Gill's Hämalaun, HE and Acridin Orange stains)
- 2 X/Y labelled cells
- 2 The MultiCap system

Information

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