

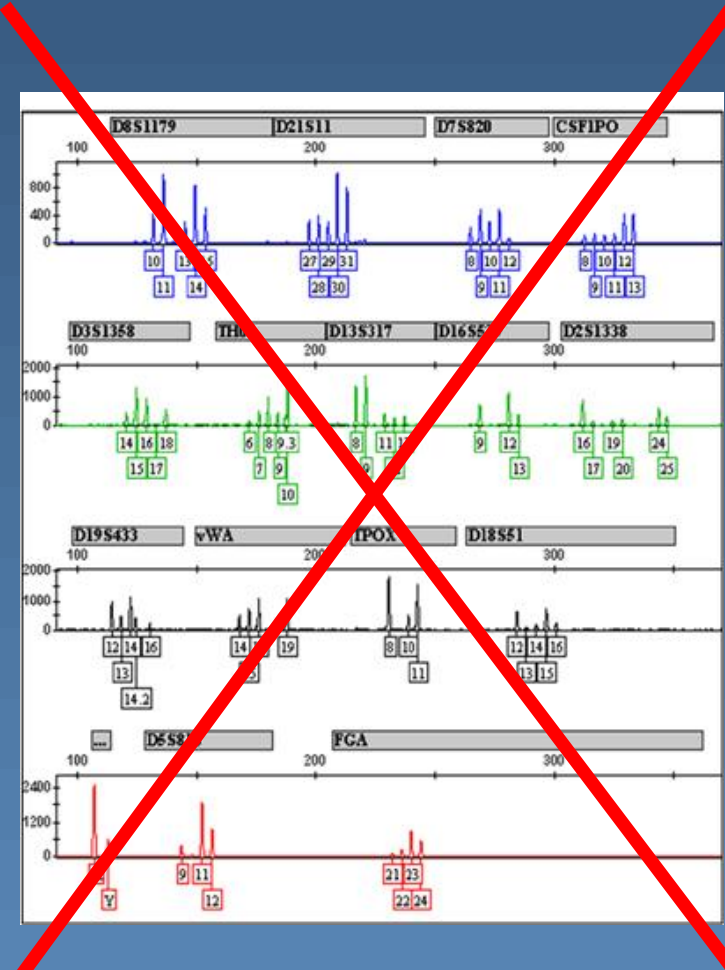
# Laser Capture Microdissection in Forensics

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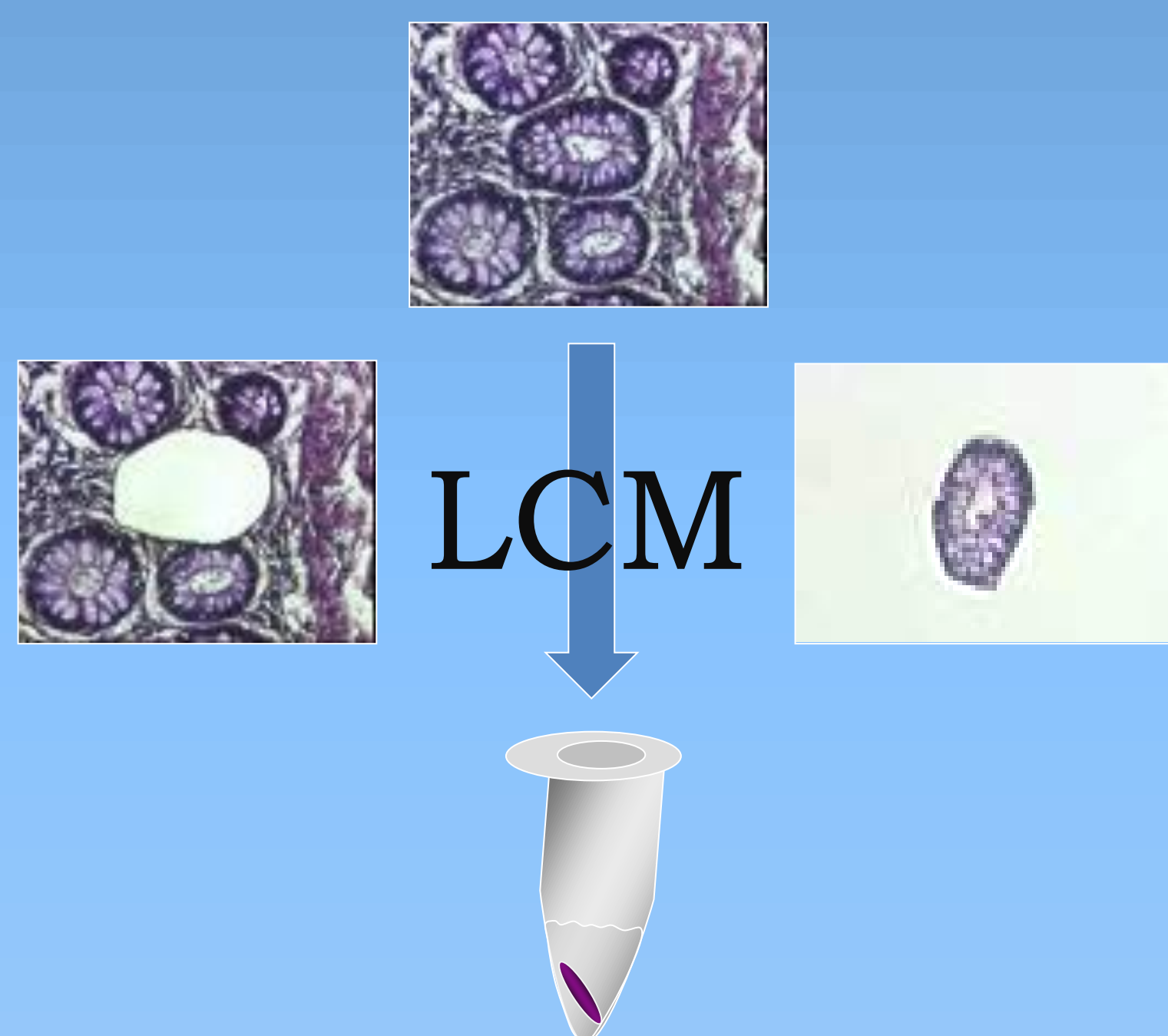
LCM system



No more mixture interpretation

## Introduction

Laser capture microdissection (LCM) is one of the most promising cell separation techniques for forensic purposes. It combines microscopy with laser beam technology and allows targeting of specific cells from traces containing cells from more than one individual, this way avoiding challenging mixture interpretation.



Before LCM can be applied, the cells of interest need to be identified based on their morphology or based on a specific staining. Staining protocols can however lead to degradation of the DNA inside the cells. Therefore, it is important to find the correct balance between discriminatory power and influence on DNA quality. Another important challenge lies in the development of a DNA extraction protocol appropriate for laser microdissected cells.

## Comparison of DNA extraction methods

3 different DNA extraction protocols after LCM were compared: DNA IQ system (Promega), PicoPure DNA extraction (Arcturus) and a short alkaline DNA extraction method using sodiumhydroxide and Tris-hydrochloric acid. This study showed that the DNA IQ system is suitable for high amounts of cells but is inadequate for lower amounts. When 200 cells were isolated, ~20% of the expected amount of DNA was recovered. This percentage drops to ~15% when 100 cells were isolated. The short alkaline DNA extraction method, which is a useful and cheap method for rapid DNA extraction of buccal swabs, is not appropriate for DNA extraction after LCM. Even isolation of 200 cells resulted in DNA profiles with significant allelic drop out. The PicoPure DNA extraction method was useful for recovery of DNA from every sample. DNA quantification showed that between 70 and 90% of the expected amount of DNA was recovered using this method.

To determine the minimal number of cells detectable by STR amplification, between 20 cells and a single cell were microdissected, followed by PicoPure DNA extraction and DNA analysis using the Profiler Plus kit (Applied Biosystems). Full DNA profiles were obtained when 10 cells or more were isolated. When fewer cells were isolated, allelic drop out could occur, but even then, useful DNA profiles were obtained. A full DNA profile could even be obtained from a single cell. These data show that LCM has a very high potential for forensic purposes.

## References:

- Vandewoestyne et al., Int J Legal Med; 2009; 123: 169-175
- Vandewoestyne et al., Int J Legal Med; 2009; 123: 441-447
- Vandewoestyne et al., Int J Legal Med; 2010; 124: 513-521
- Vandewoestyne et al.; Forensic Sci Int Genet, 2012;6(2):258-262

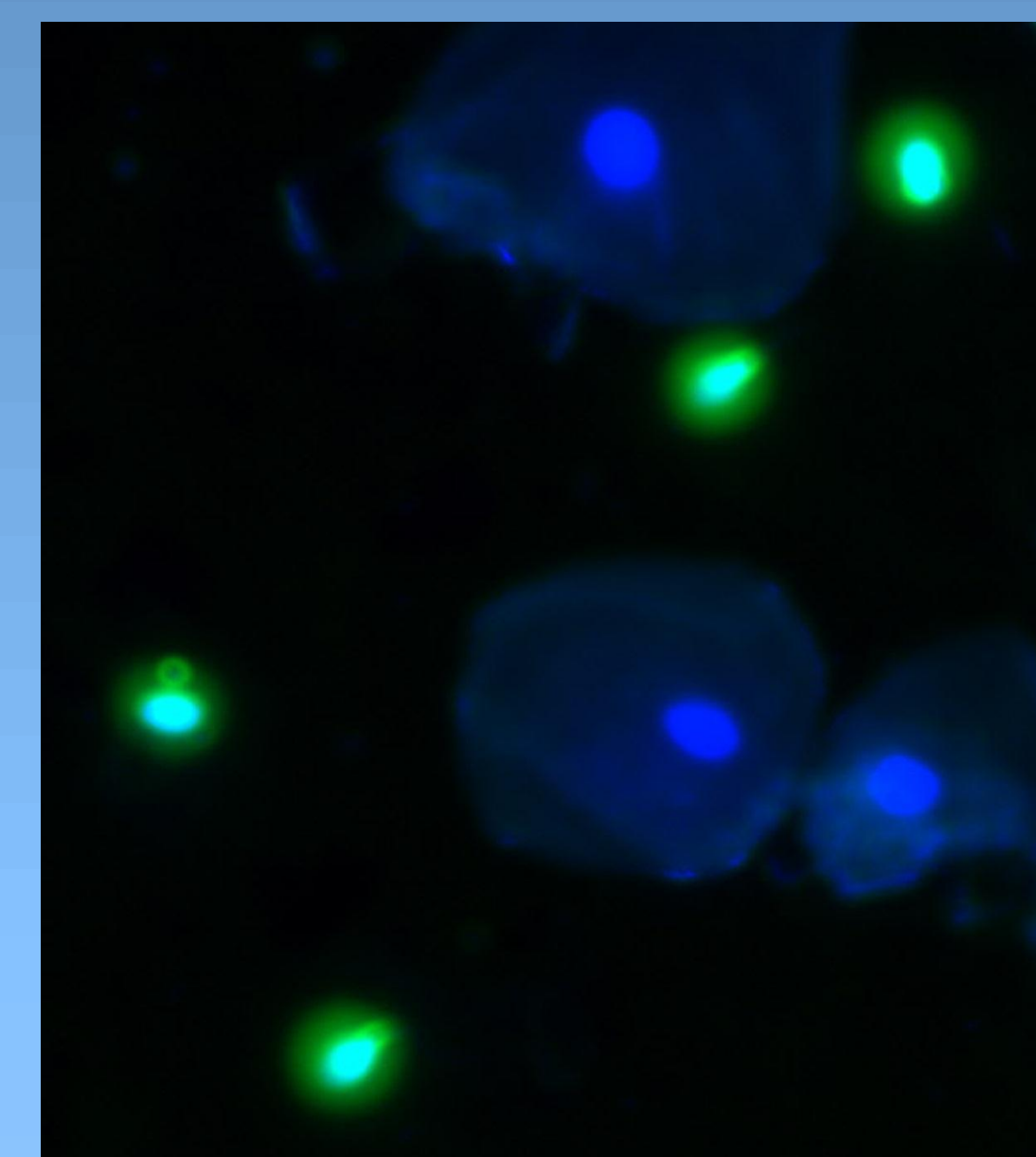
N° of cells	Expected DNA yield	DNA IQ System		Short alkaline method		PicoPure	
		Profile recovery	DNA yield	Profile recovery	DNA yield	Profile recovery	DNA yield
200	1320 pg	+++	279 pg	-	<detection limit	+++	892 pg
100	660 pg	+	96 pg	--	<detection limit	+++	464 pg
50	330 pg	-	<detection limit	--	<detection limit	+++	308 pg
25	165 pg	--	<detection limit	--	<detection limit	+++	128 pg
0	0 pg	---	<detection limit	---	<detection limit	---	<detection limit

N° of cells	Exp. 1: # allelic drop outs	Exp. 2: # allelic drop outs	Exp. 3: # allelic drop outs
20	0	0	0
10	0	0	0
5	0	1	0
1	3	0	4

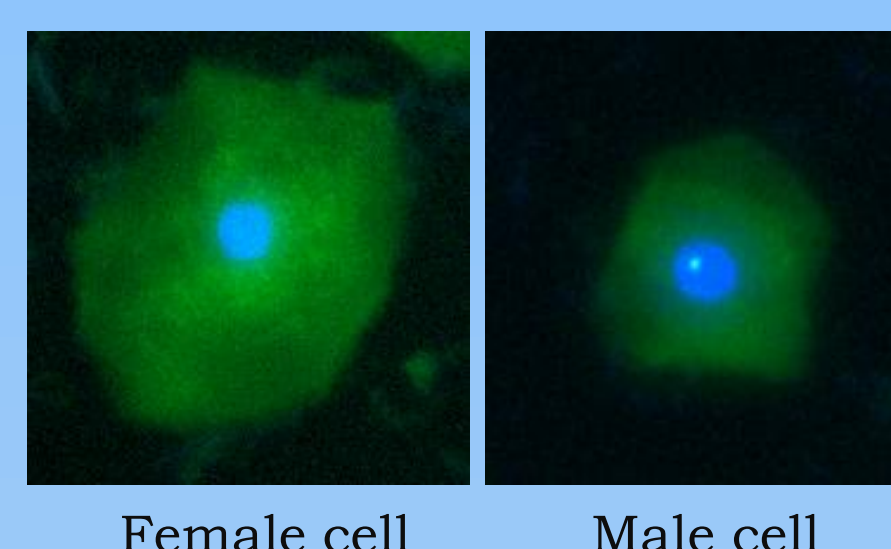
## Forensic applications of laser capture microdissection

Up til now, LCM has mainly been used to isolate sperm cells from sexual assault samples. The search for sperm cells on a microscope slide by visual inspection is very time-consuming and labour-intensive, especially for slides containing a low number of sperm cells.

Therefore, the slides are stained with Sperm HyLiter (Independent Forensics), combining fluorescently labeled monoclonal antibodies directed against a protein contained in the human sperm head and a DAPI staining of all nuclei present on the slide. The detected sperm cells can then be collected by a laser pulse and are used for DNA profiling.



Vaginal smear stained with Sperm Hy-Liter



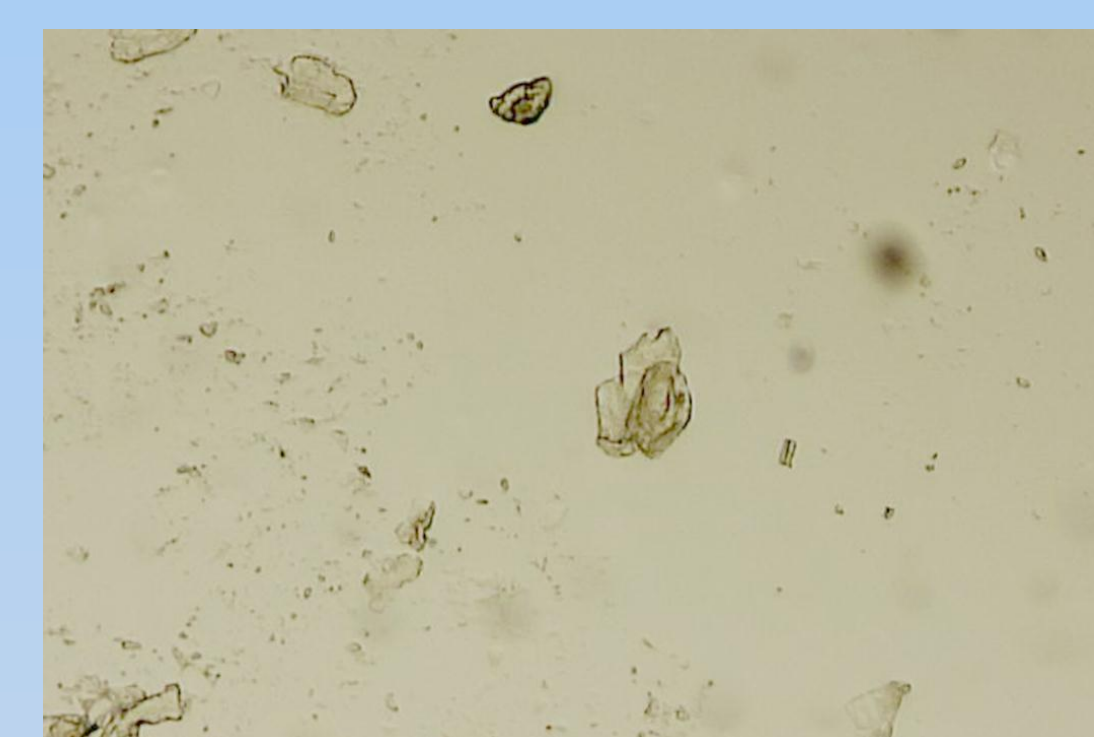
Female cell

Male cell

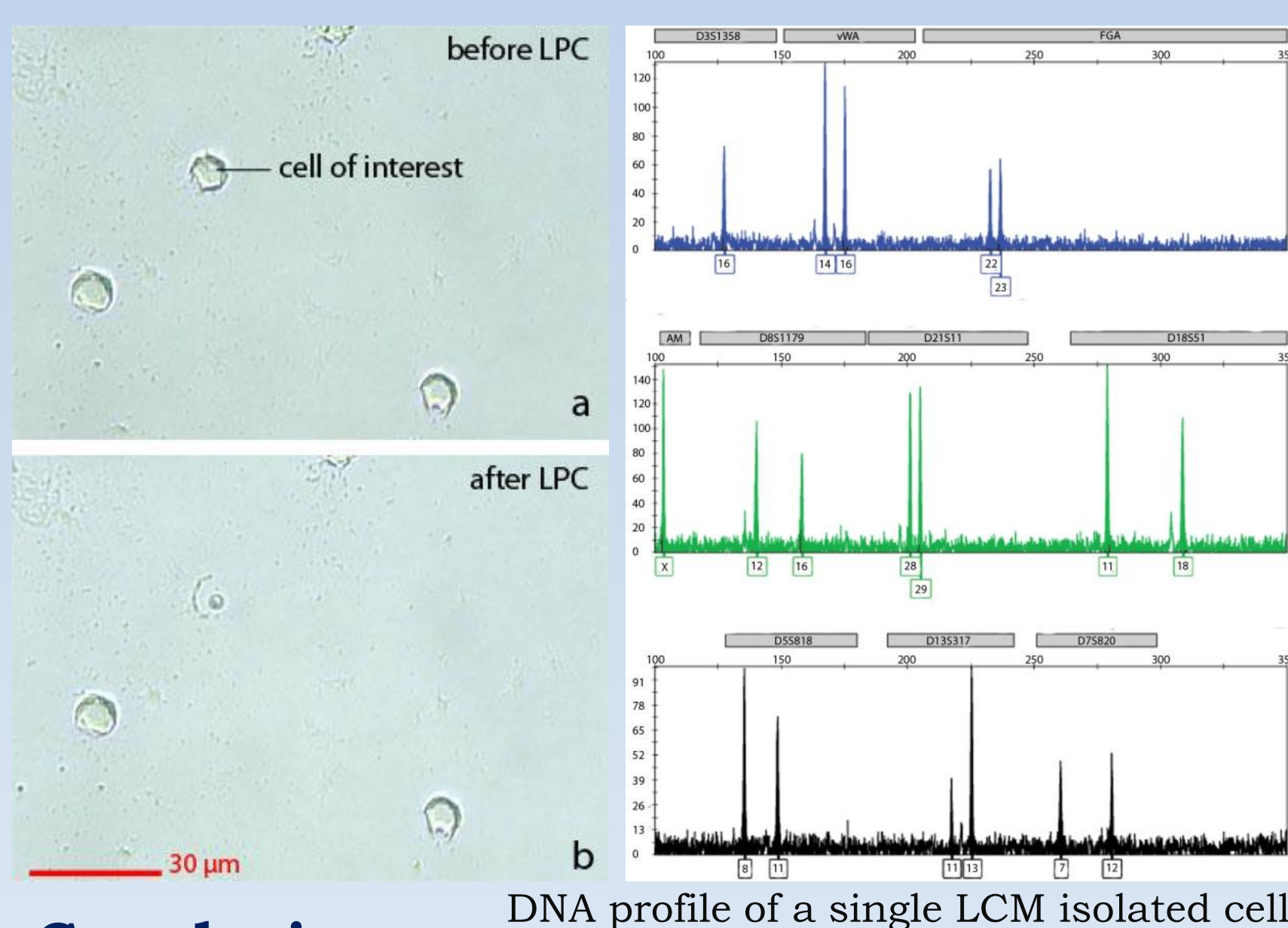
When there is no ejaculation or when the rapist was a sterile man, male, non-sperm cells can be found on the victim's body. Male and female cells are distinguished using fluorescence in situ hybridization (FISH) with a Y-chromosome specific probe.

Although most studies have been concentrating on the use of LCM in sexual assault cases, it can also be used in other forensic applications. LCM can be very useful to isolate single hair follicles, to perform a more efficient DNA extraction and amplification, without the presence of PCR inhibitors such as keratin.

LCM can also be used to isolate cells present on adhesive tape, used for sampling in cars or on corpses. Profile recovery depends on the cell type that has been isolated. Skin cells, for example, may have lost their nucleus due to apoptosis and DNA degradation during keratinisation of the skin. Therefore, no exact statement can be made on how many skin cells need to be isolated to obtain a useful DNA profile.



Epithelial cells on adhesive tape



DNA profile of a single LCM isolated cell

## Conclusions

LCM is a very specific cell separation technique for the isolation of pure cell populations. The fact that routine glass slides can be used offers an enormous advantage, as it can also be performed on archived material. As it is a very versatile technique, applicable on all kinds of sample types, it has an unlimited range of applications. The use of LCM should therefore be considered in every forensic case where mixture separation is desirable.