Detection and Analyses of Latent DNA

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Human Identification Solutions
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Thanks to:

Piyamas Kanokwongnuwut
Belinda Martin
&
Renée Blackie

Deposition of 'Touch' DNA

Touching an item for only a few seconds can leave very little DNA

The DNA deposited by contact is invisible and at trace levels

The amount deposited by a very brief contact, or deposited in the past and degraded by bacterial growth, may no longer be suitable for standard DNA profiling.

Standard Current Process

These types of items are routinely submitted for forensic analysis

"*Who touched this last?*"

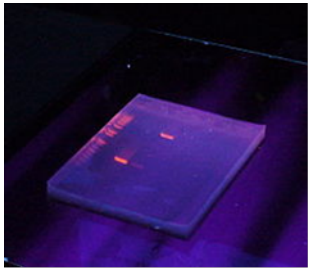
Evidence recovery teams will make an educated assumption as to where to sample

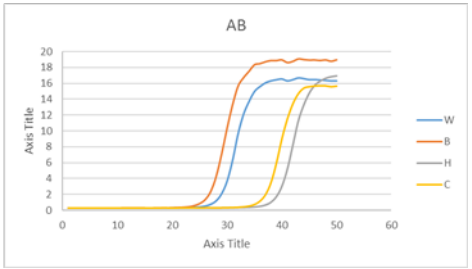
The back of a cartridge case is a defined area but the whole length of a knife handle is more of an issue

Latent DNA Detection

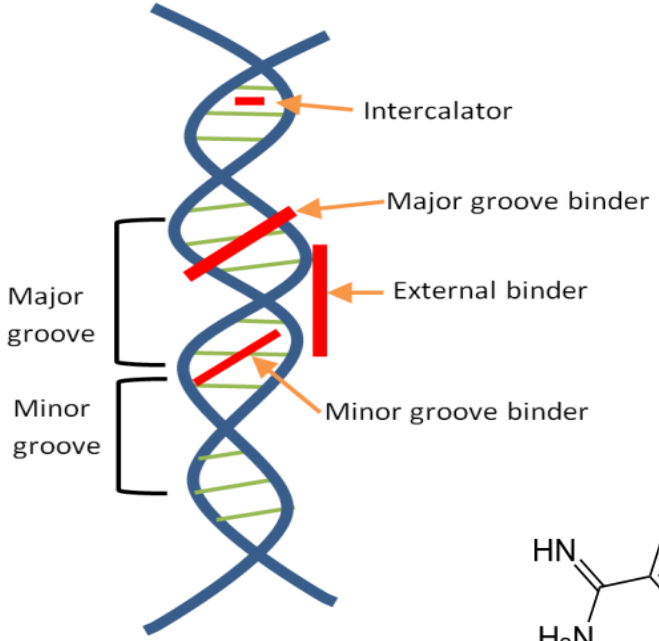
Dyes detect DNA in gels and in real-time PCR so why not on items?

If dyes can detect and ‘visualise’ the presence of DNA then can target the collection of the DNA.

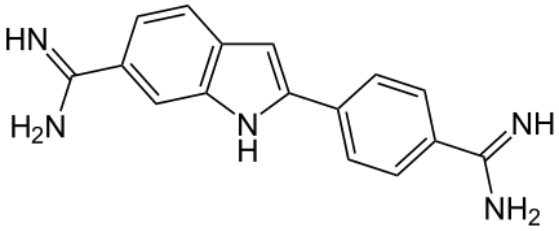




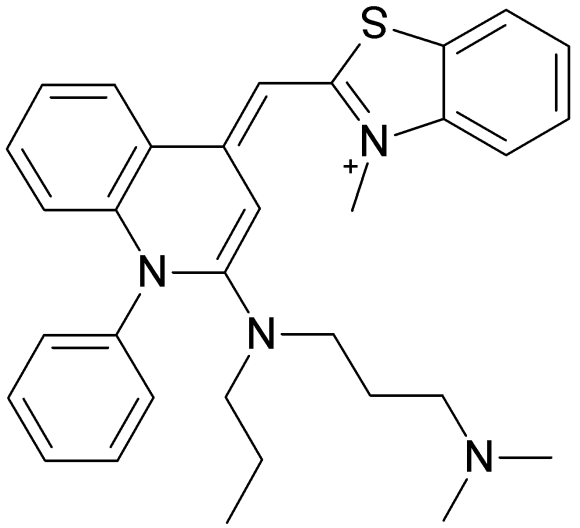
Nucleic Acid Binding Dyes



DAPI



SYBR® Green I



Diamond Dye

Groove Binding dyes

Intercalating Dyes

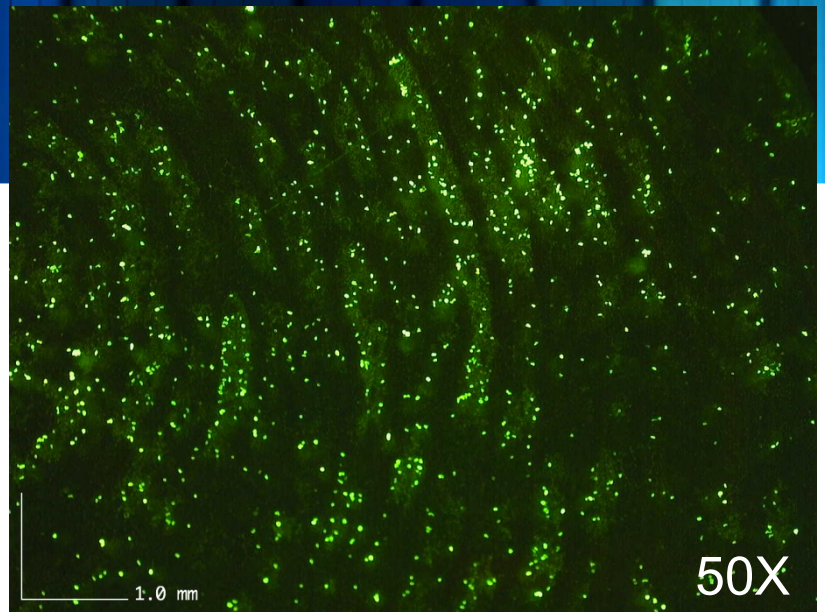
Experimental Process

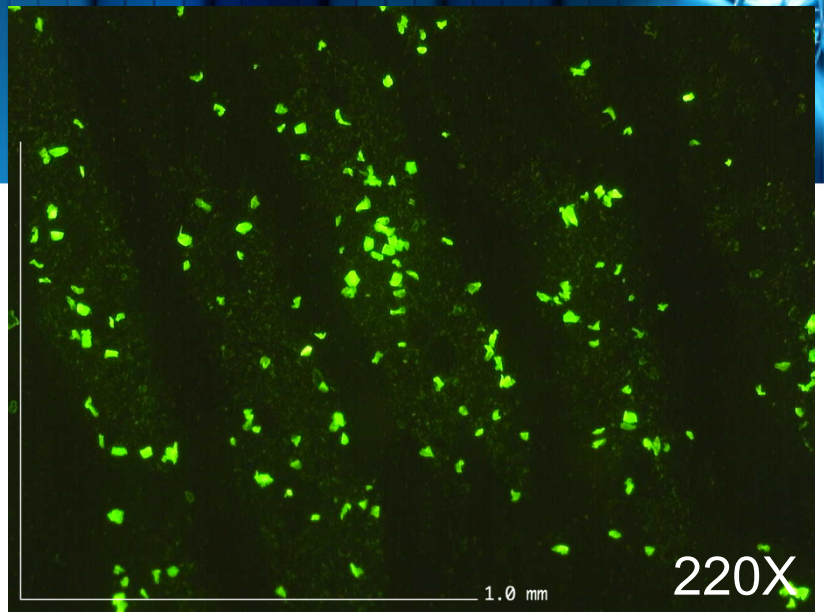
A range of volunteers of varying shedder status were used.

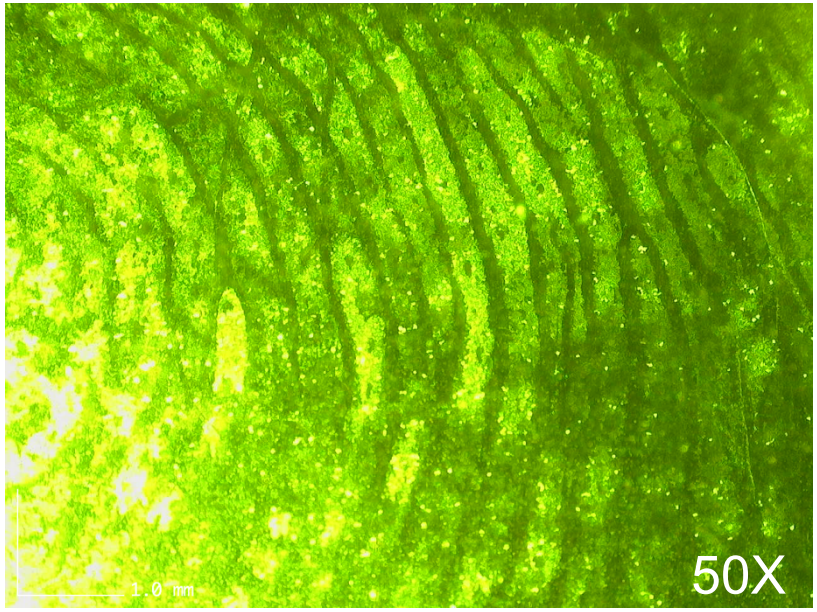
All washed and dried hands. Touched items at specific times points starting at time 0, and then 15, 30, 60, 180 minutes after handwashing.

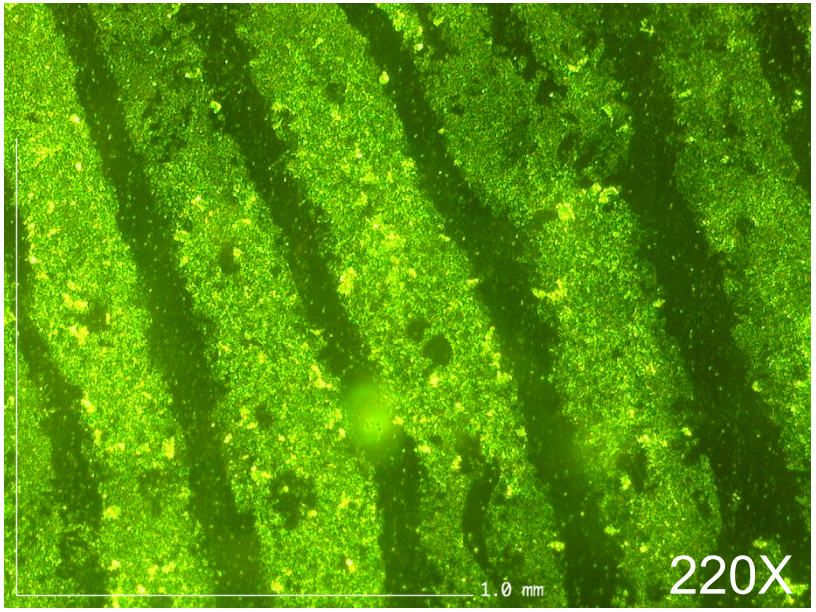
All touching was for 15 seconds or less and all performed in triplicate

Fingermark

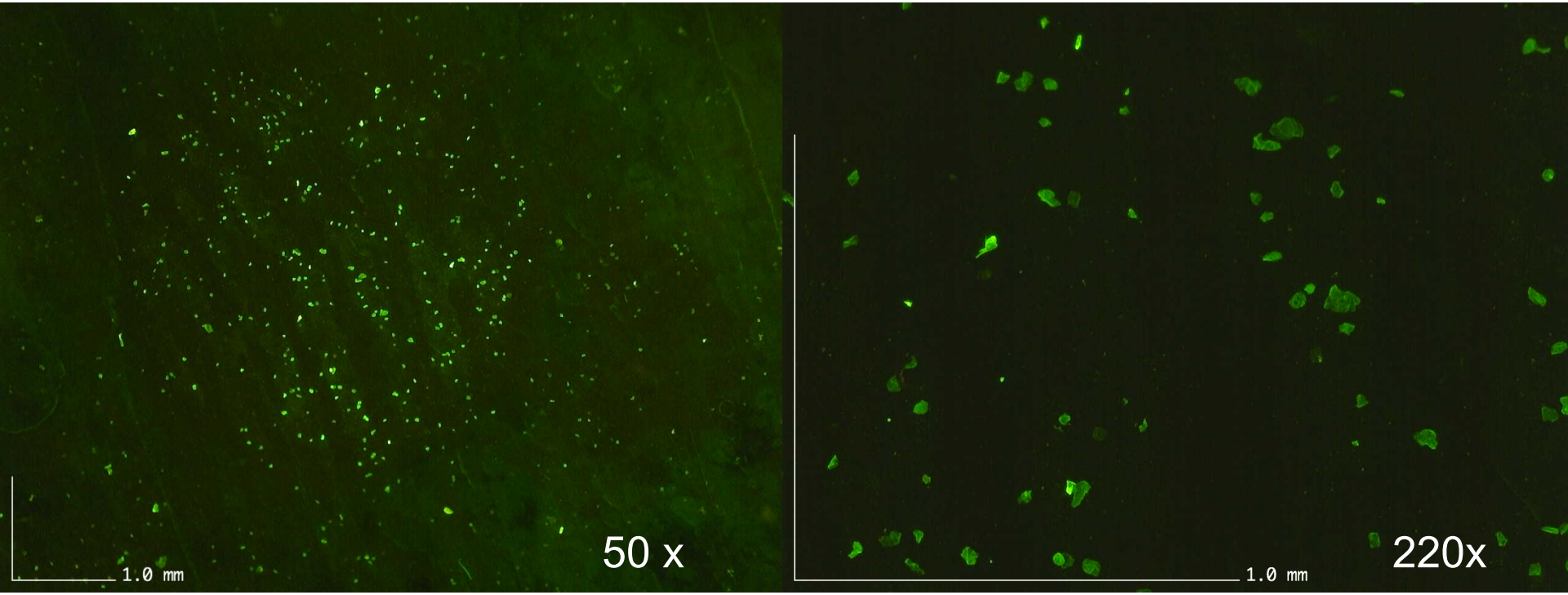








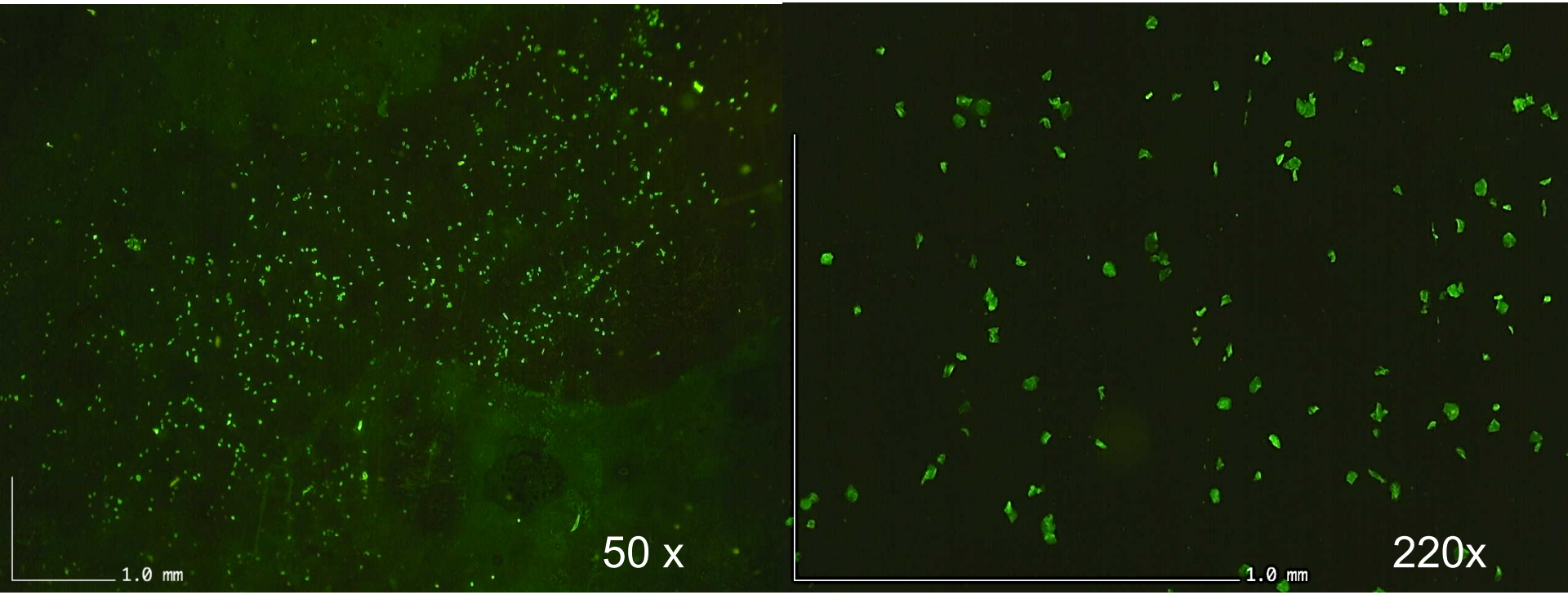
Detecting DNA in a Fingermark



Right thumb

Time : 15 min

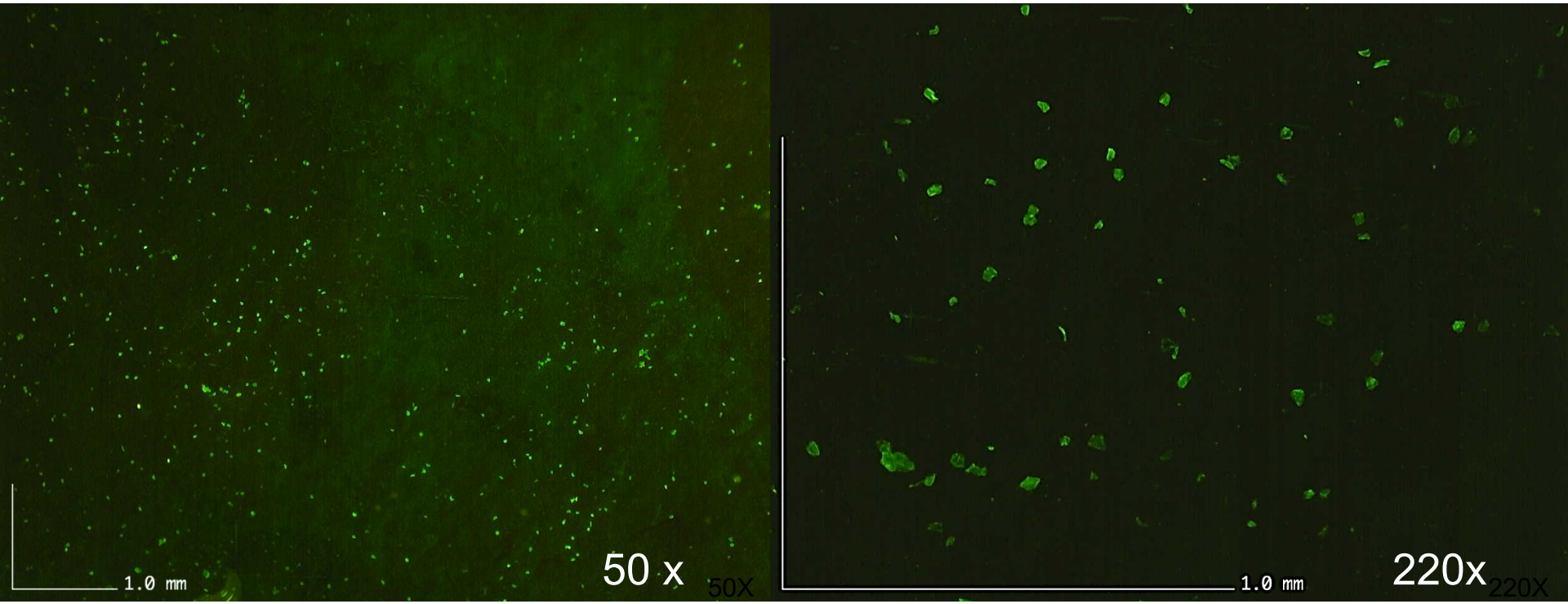
Detecting DNA in a Fingermark



Right thumb

Time : 1 h

Detecting DNA in a Fingermark

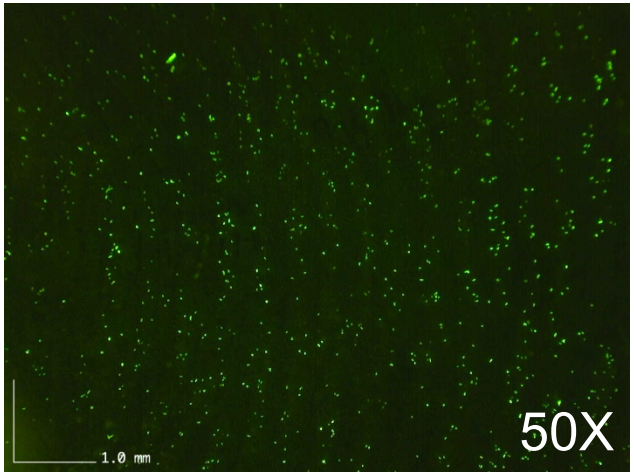


Right thumb

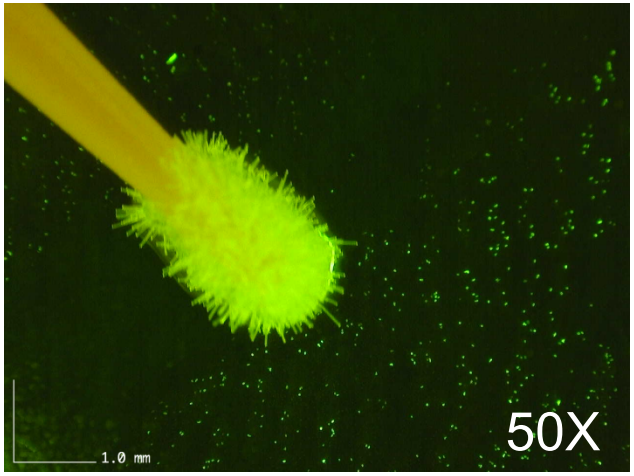
Time : 3 h

Collection of Biological Material

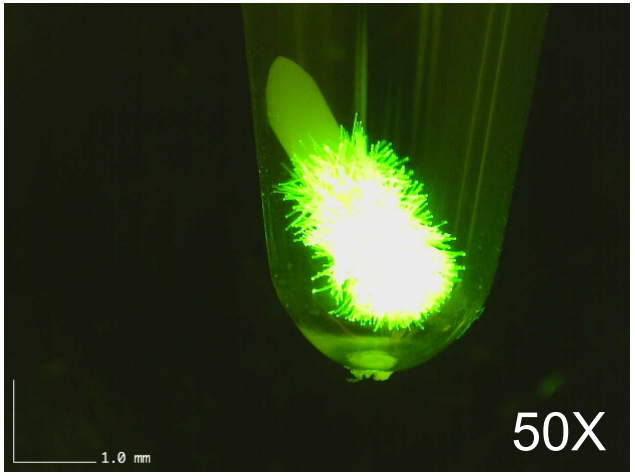
Stain



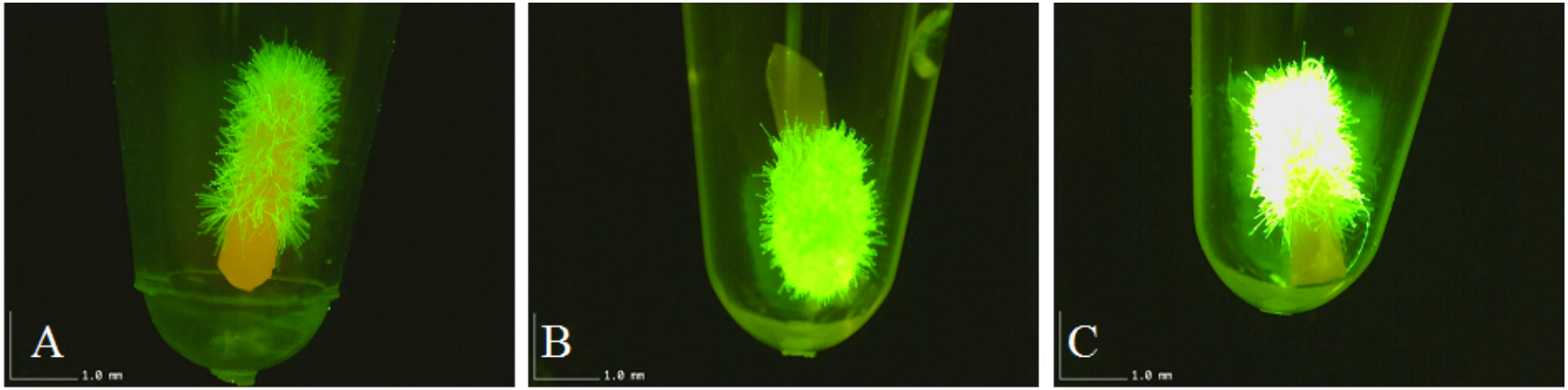
Swab



Direct PCR

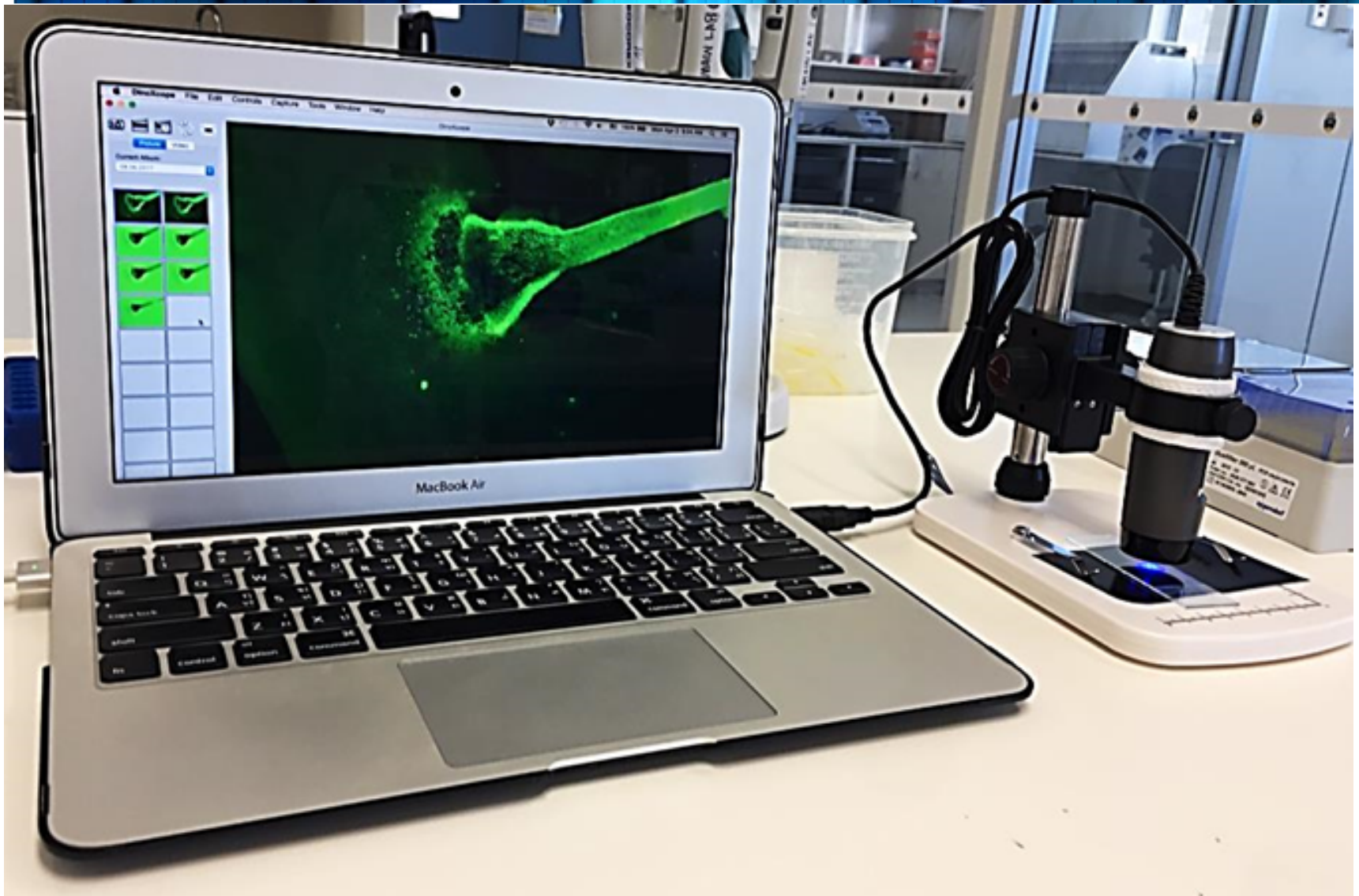


DNA Transfer to Swab

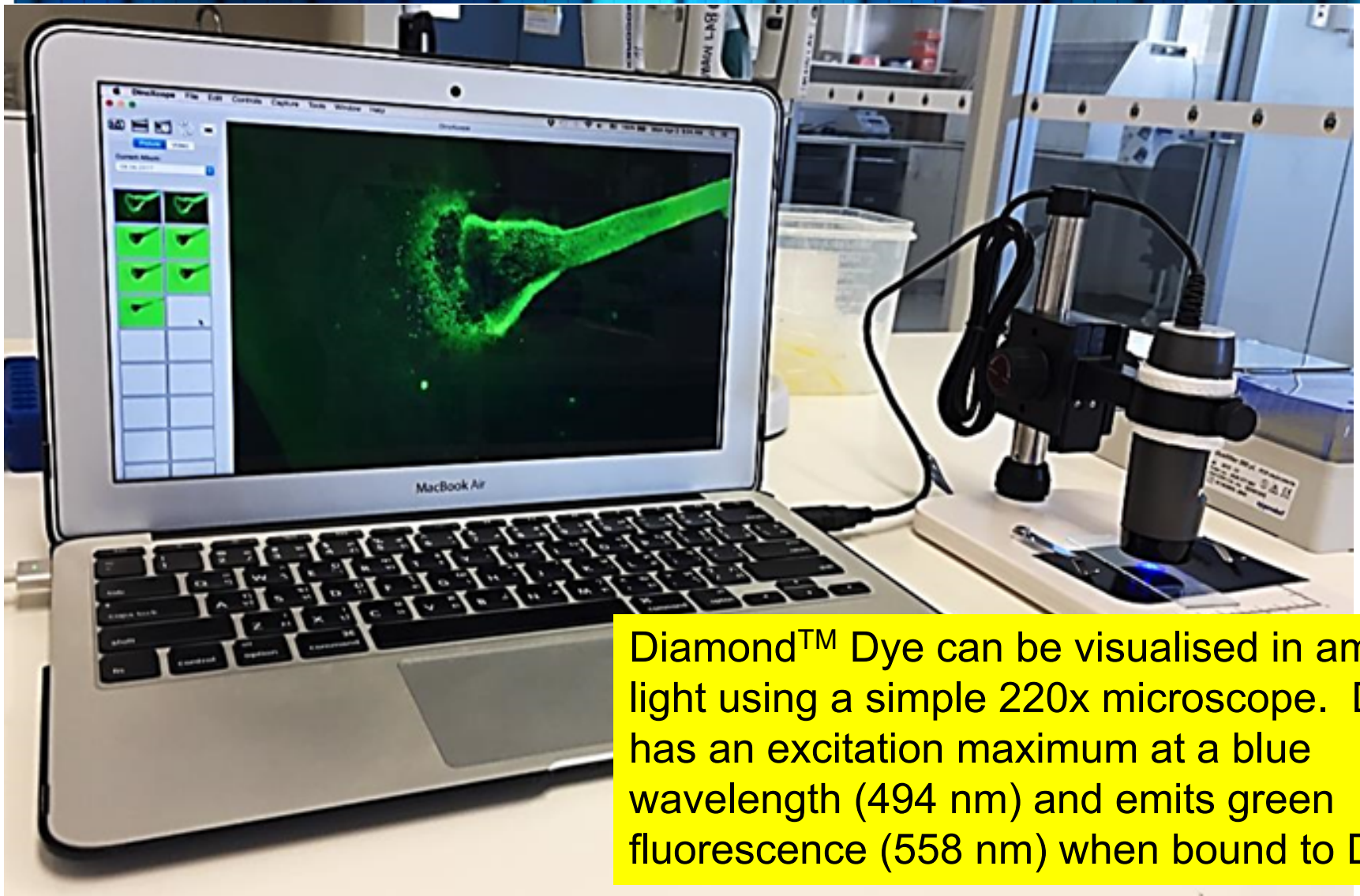


(A) a non-stained swab, (B) a swab stained with Diamond™ Dye before collecting any cellular material and (C) a swab stained with Diamond™ Dye after the collection of cellular material

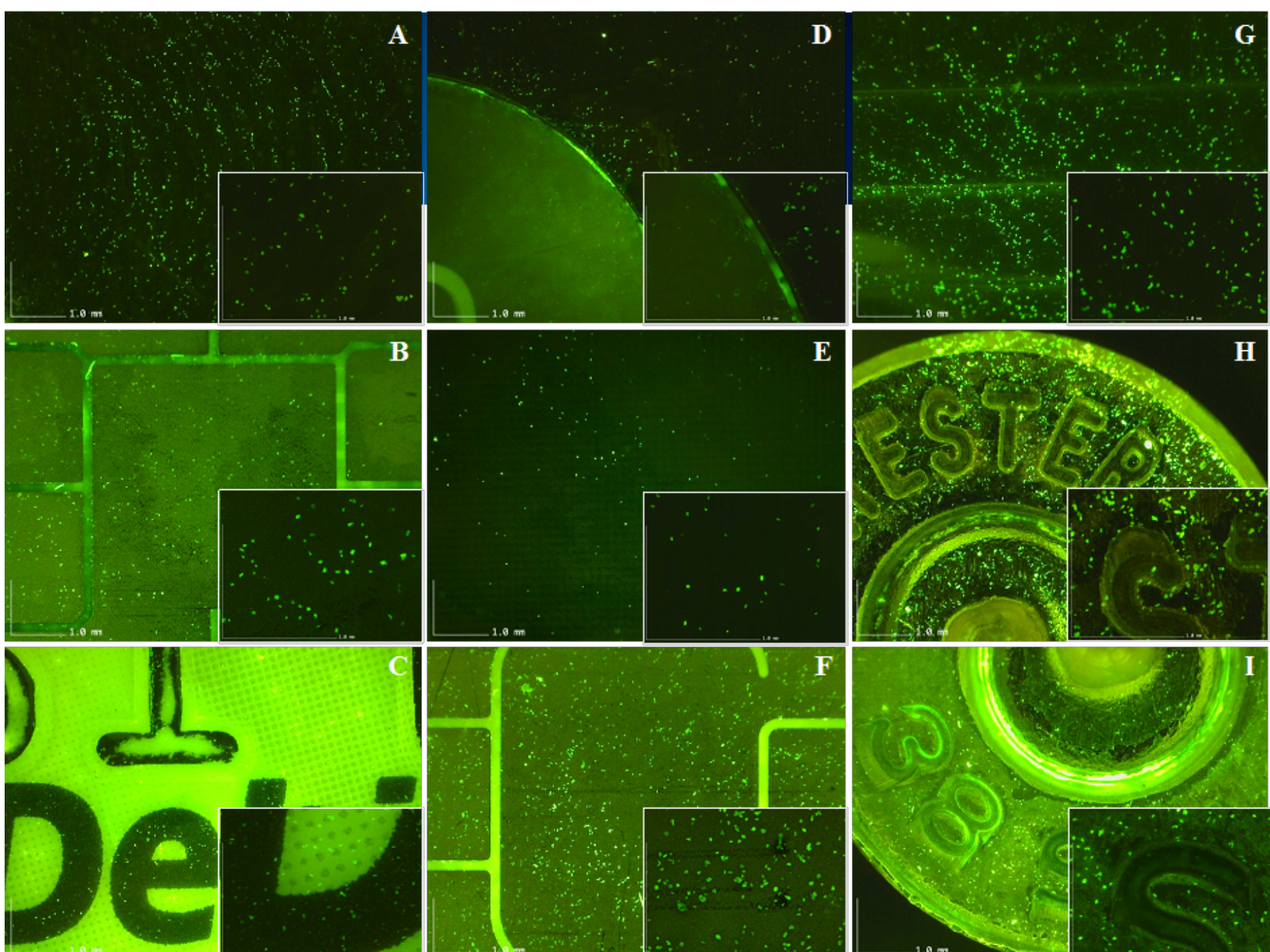
Simple equipment needed



Simple equipment needed

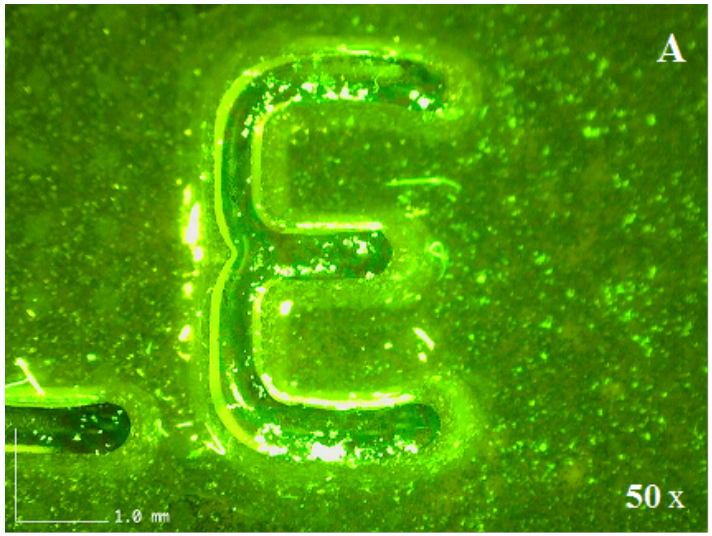


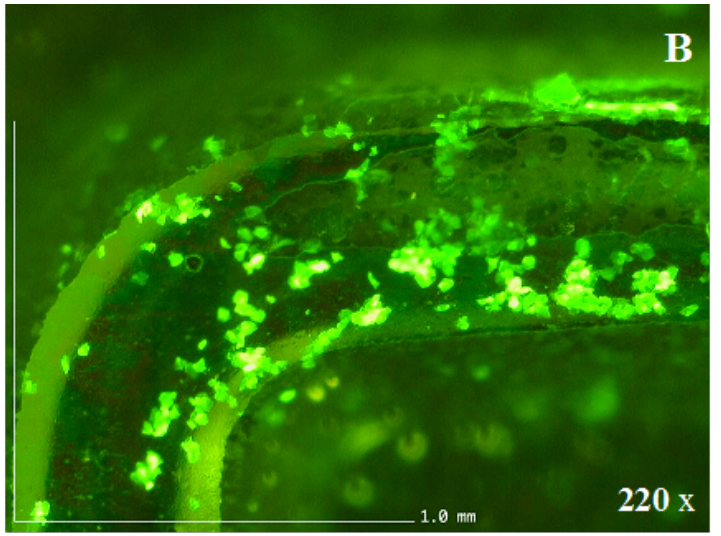
Diamond™ Dye can be visualised in ambient light using a simple 220x microscope. DD has an excitation maximum at a blue wavelength (494 nm) and emits green fluorescence (558 nm) when bound to DNA.

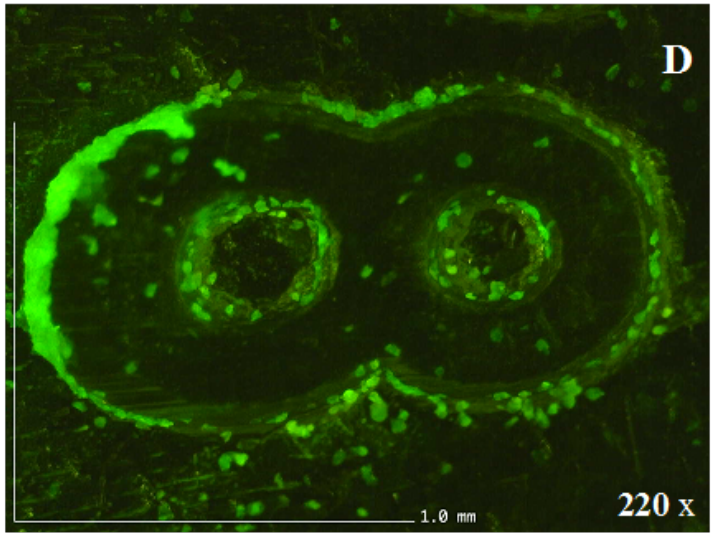

A
D
G
B
E
H
C
F
I

Credit card and catridge case

Cellular material collecting at embossed numbers on the credit card under 50 x (A) and 220 x (B) and on the head of the nickel cartridge case under 50 x (C) and 220 x (D).

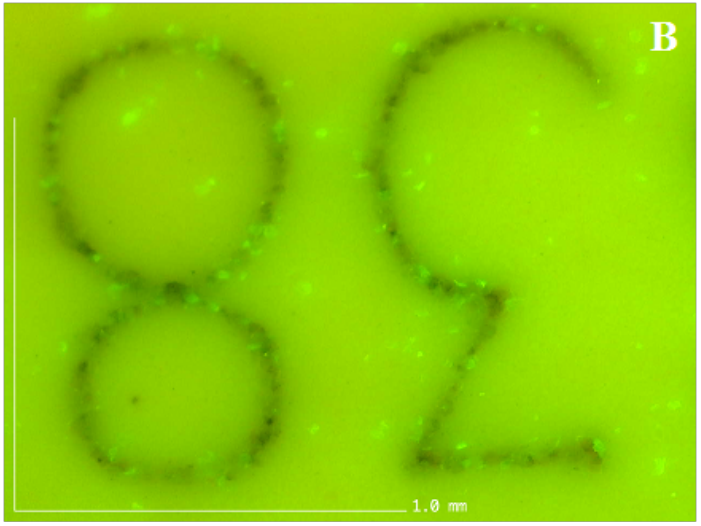


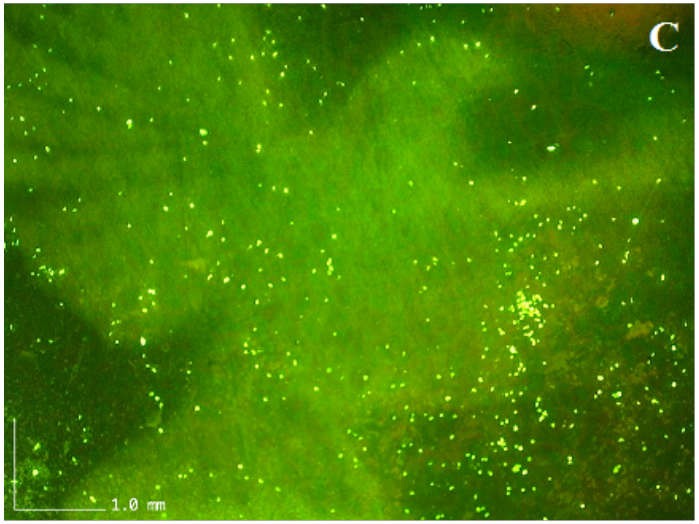


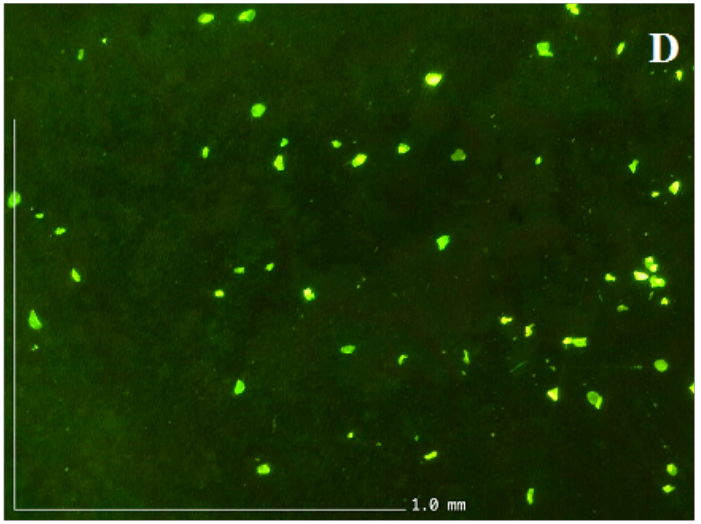


Sim Card and Credit Card

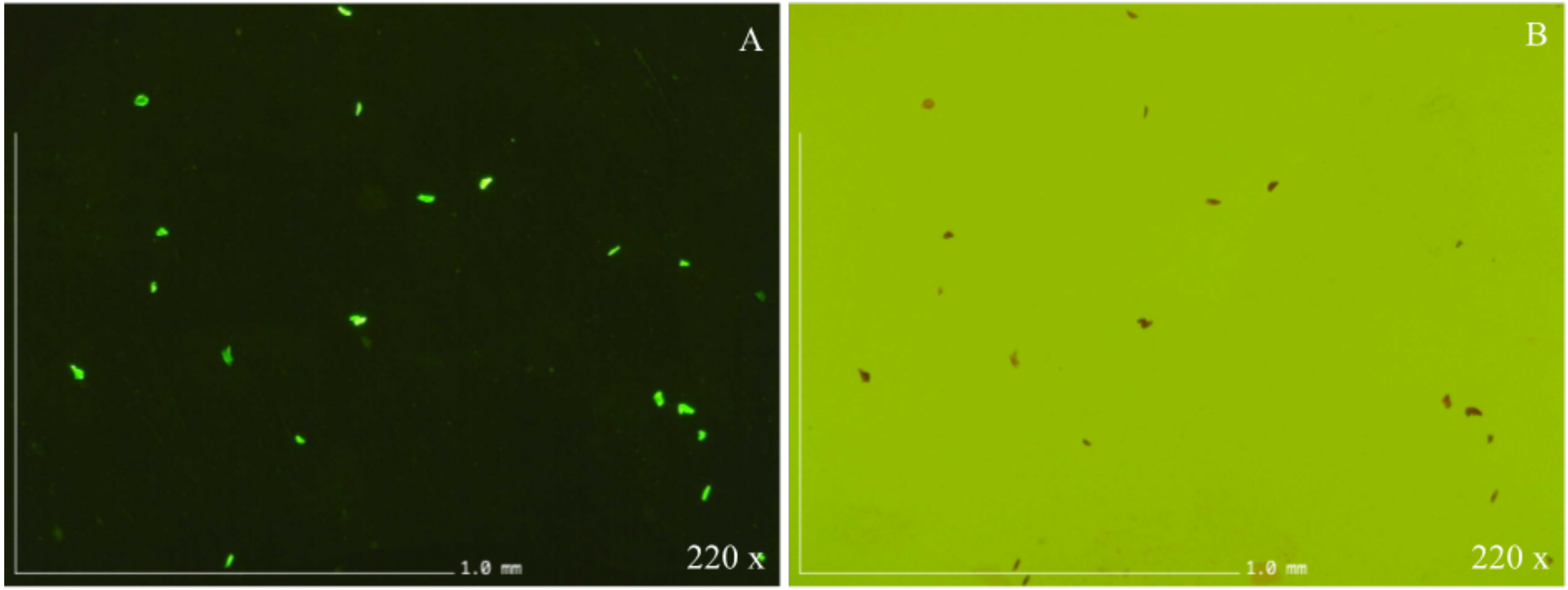
The reverse (back) side of SIM card under 50 x (A) and 220 x (B), and the reverse (back) side of credit card at the security hologram under 50 x (C) and 220 x (D).





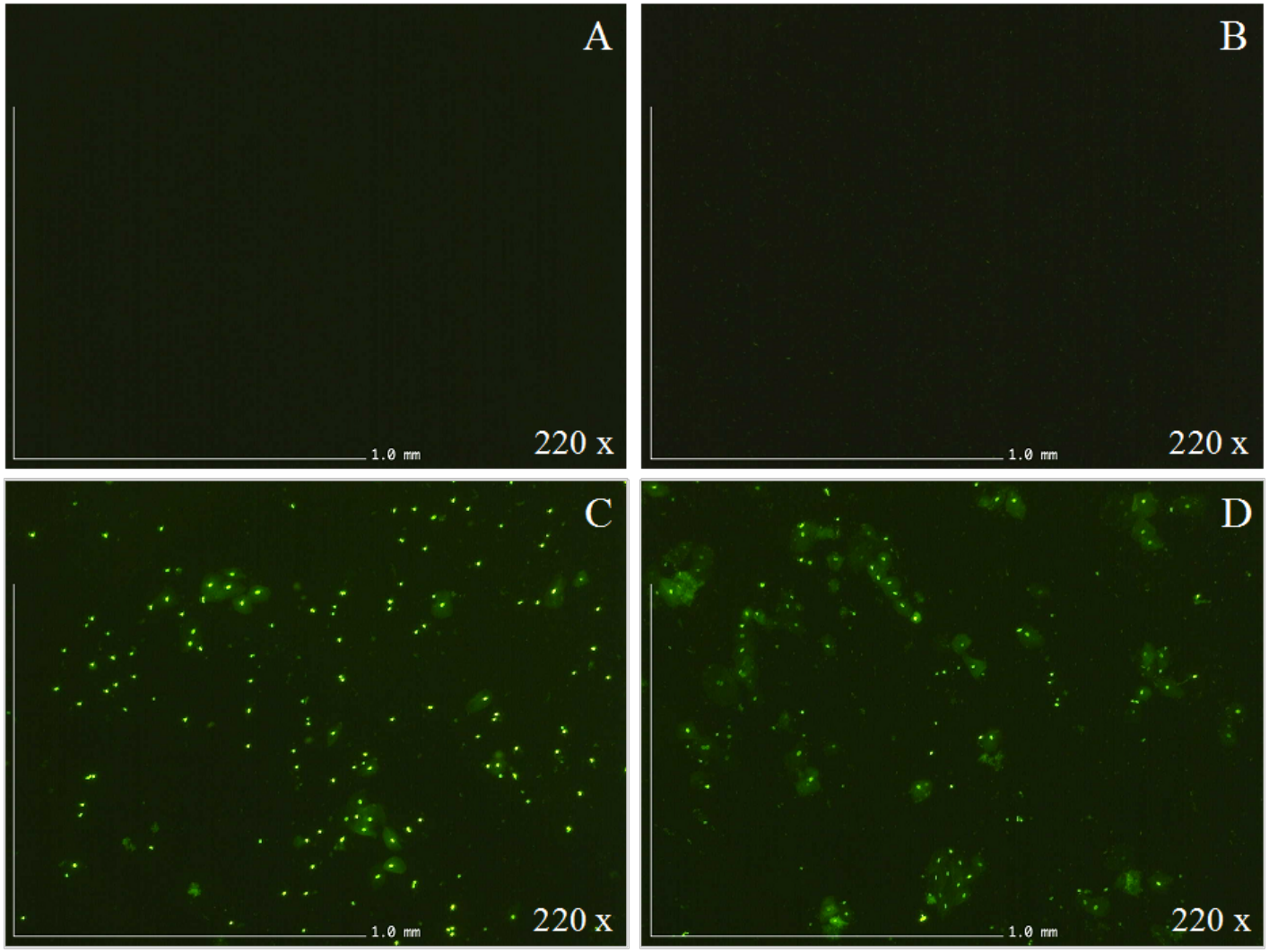


Confirmation of Cellular Material



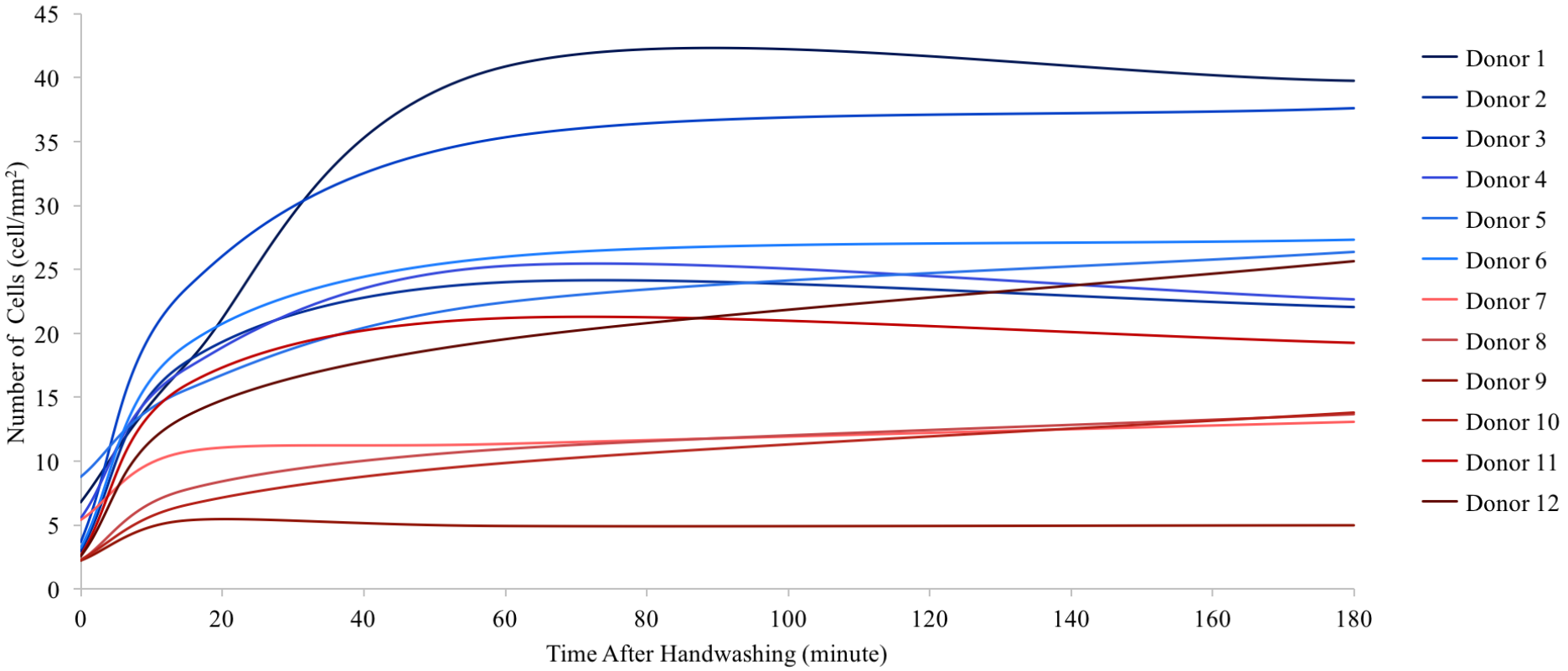
Cellular material within fingermark on a glass slide after staining with 20 x Diamond™ dye (A), haematoxylin and eosin (B) detected under the microscope 220 x magnification

Bacterial Testing (*E. coli DH5alpha*)



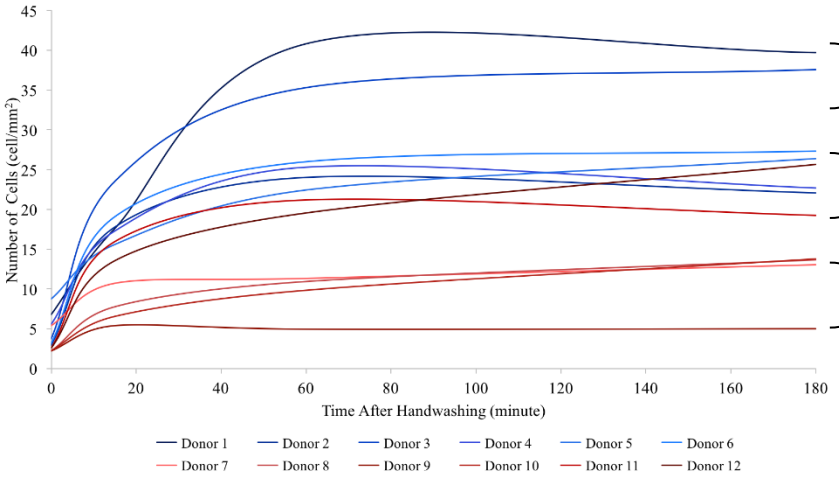
Negative control (no bacteria) (A), bacteria stained with DD (B), saliva stained with DD (C), and *E. coli* mixed with saliva (1:1) (v/v) stained with DD (D).

Speed of DNA Accummulation



12 donors washed their hands and then made contact with a clean glass slide for 15 seconds with medium pressure. The times post-handwashing were 0, 15, 60 and 180 minutes. The tests were performed in triplicate and an average shown.

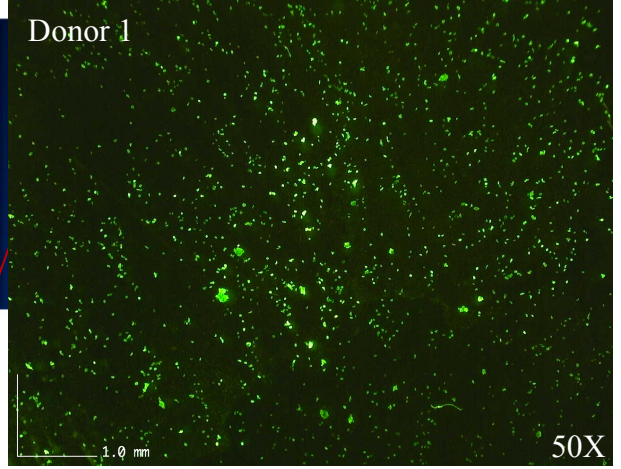
Cellular Material

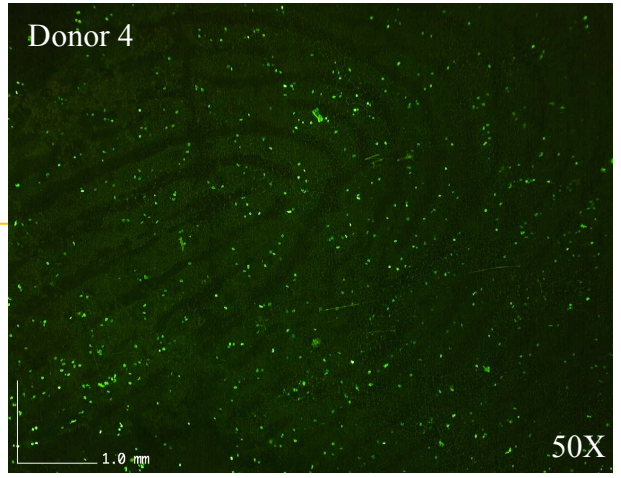


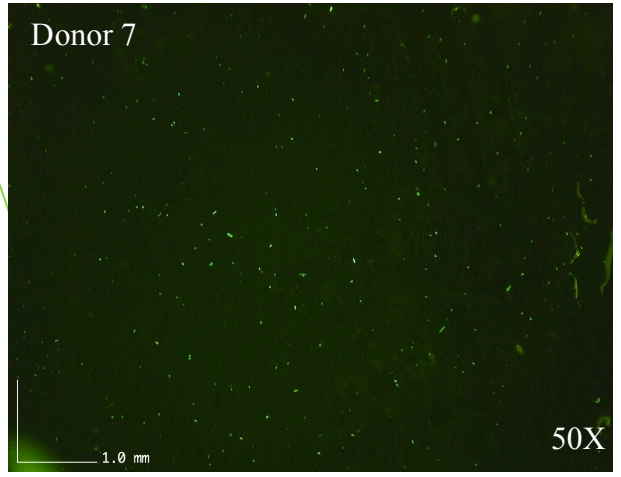
Heavy Shedder

Intermediate Shedder

Light Shedder

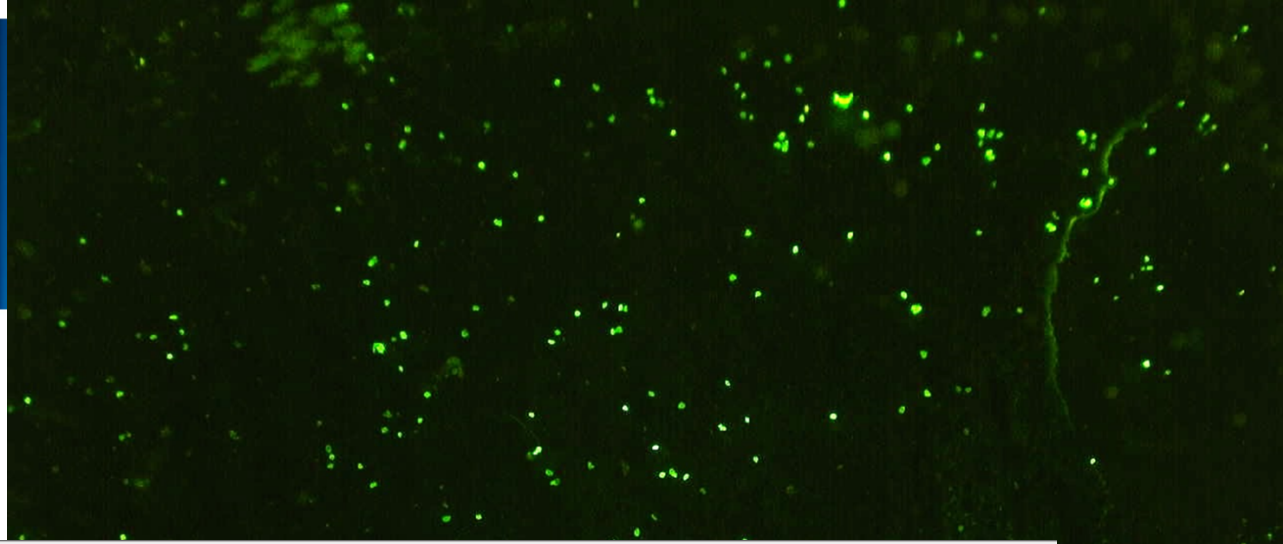


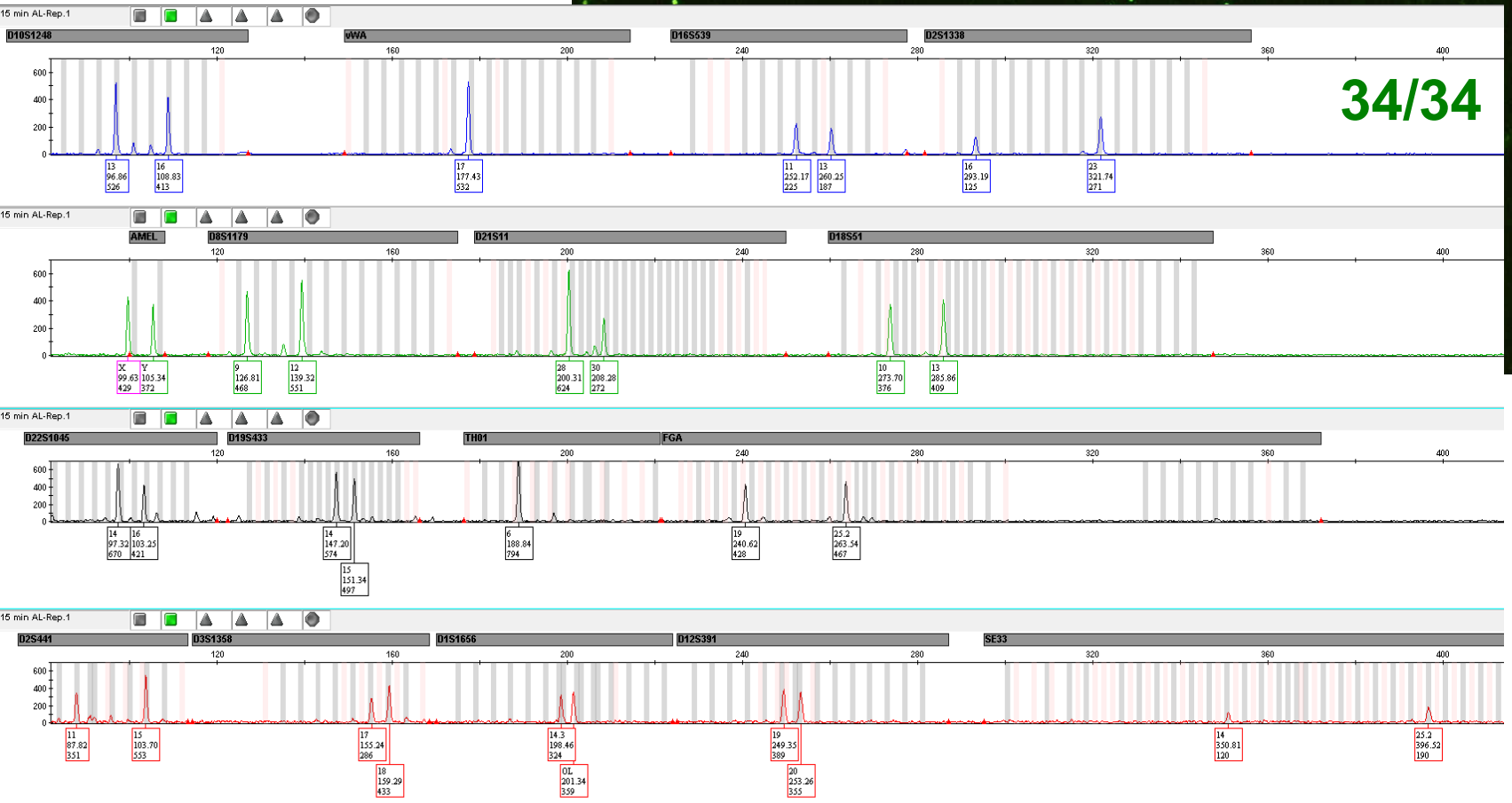




Donor 1 (15 min)

% STR : 100%



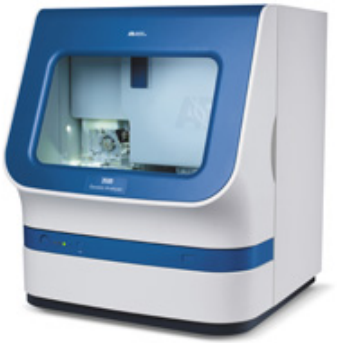


50X

Enhancement of Touch DNA

- Direct PCR is a viable alternative
  - No need to increase cycle number, fits with standard processes, now being used in casework
- Use specific micro-swabs to collect DNA
  - Place the entire swab head into the PCR

Direct PCR Forensic Process

DNA extraction	DNA quantification	PCR	CE & data analysis
			
2 hours	2-3 hours	2-3 hours	2-3 hours

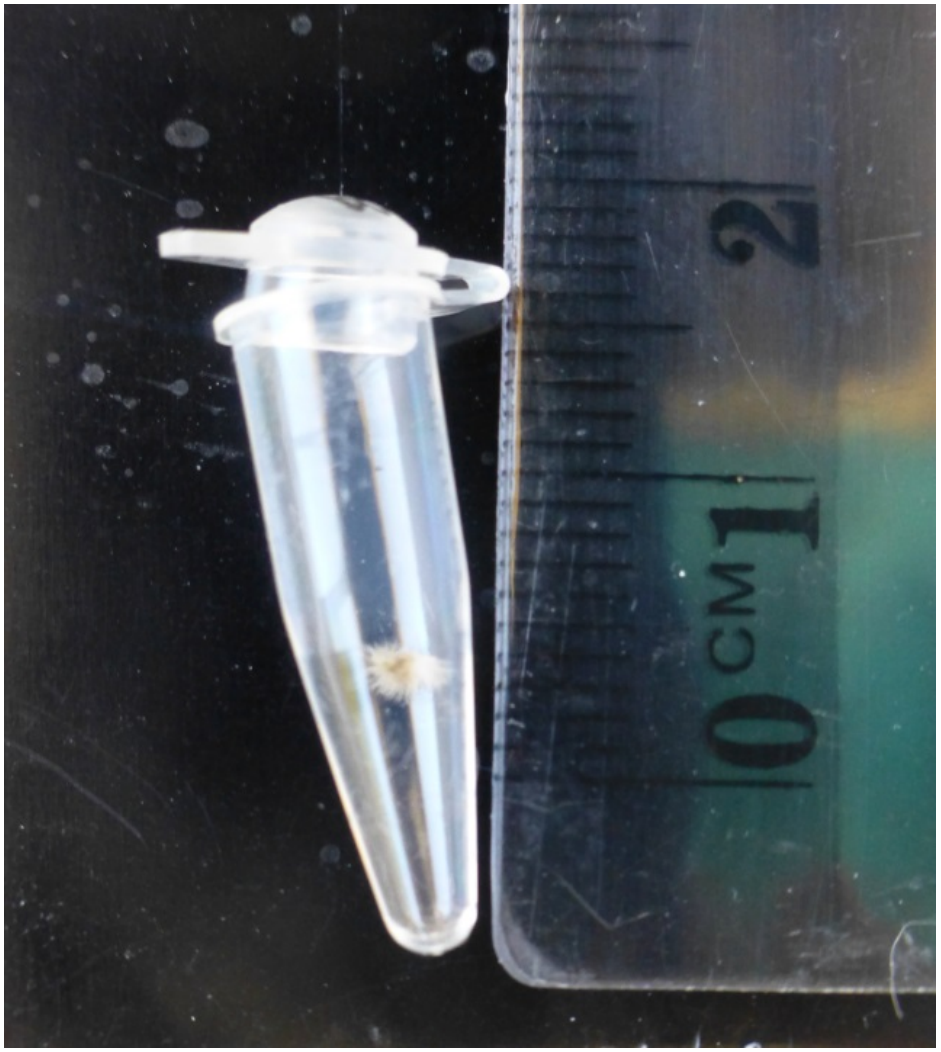
Direct PCR

We use positively charged nylon fibres to bind the DNA to the swab

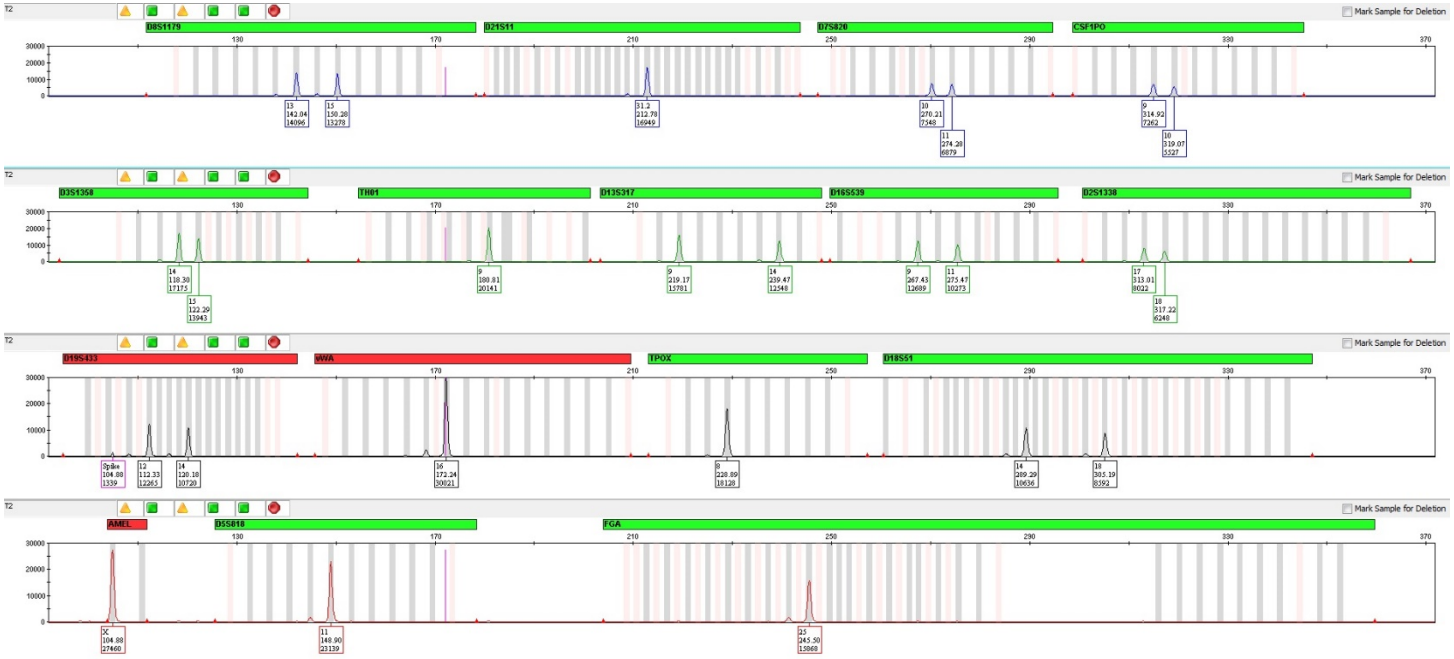
Found to be much more efficient.
But….. must be able to release the DNA in PCR

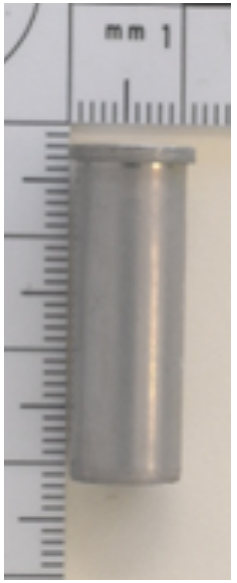
Direct PCR

Fibres removed and placed directly into the PCR tubes containing the 1 x PCR solution



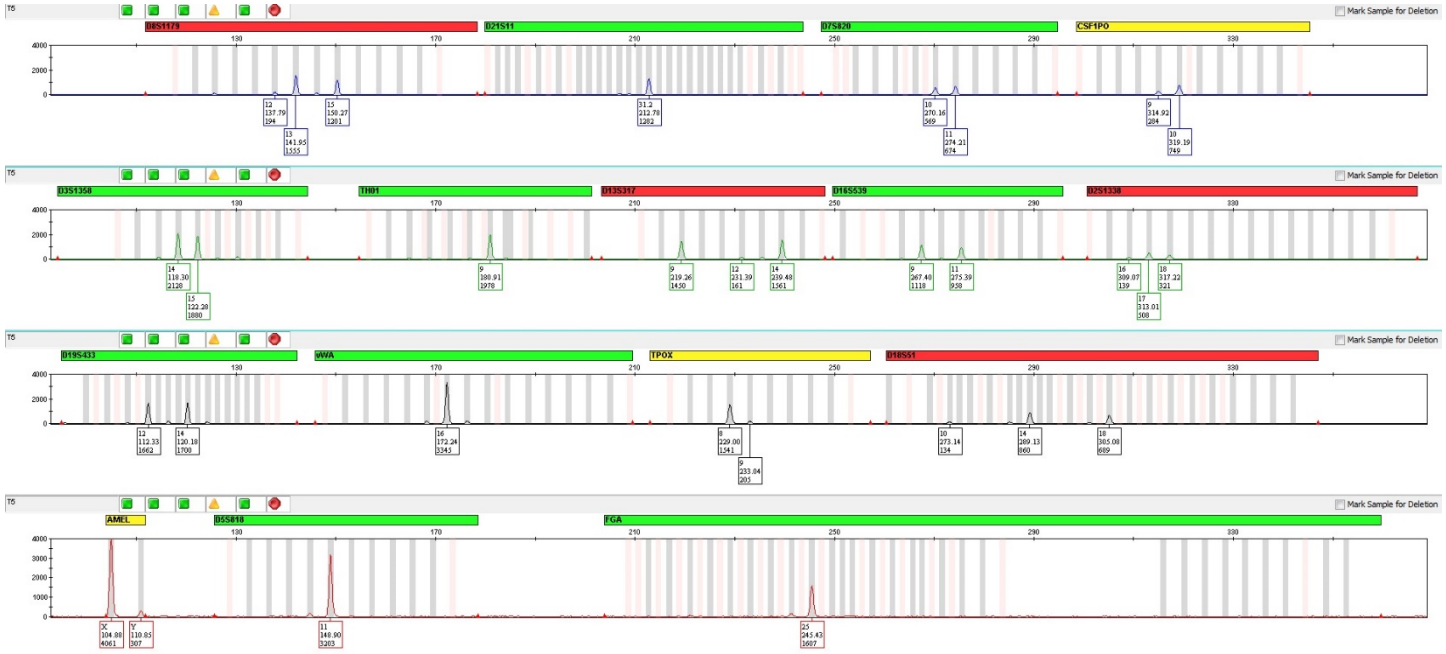
Aluminium Cartridge





Cartridge case held for 5 seconds, stained, swabbed and direct PCR using Identifiler Plus. Full profile obtained matching the person that held the cartridge case.

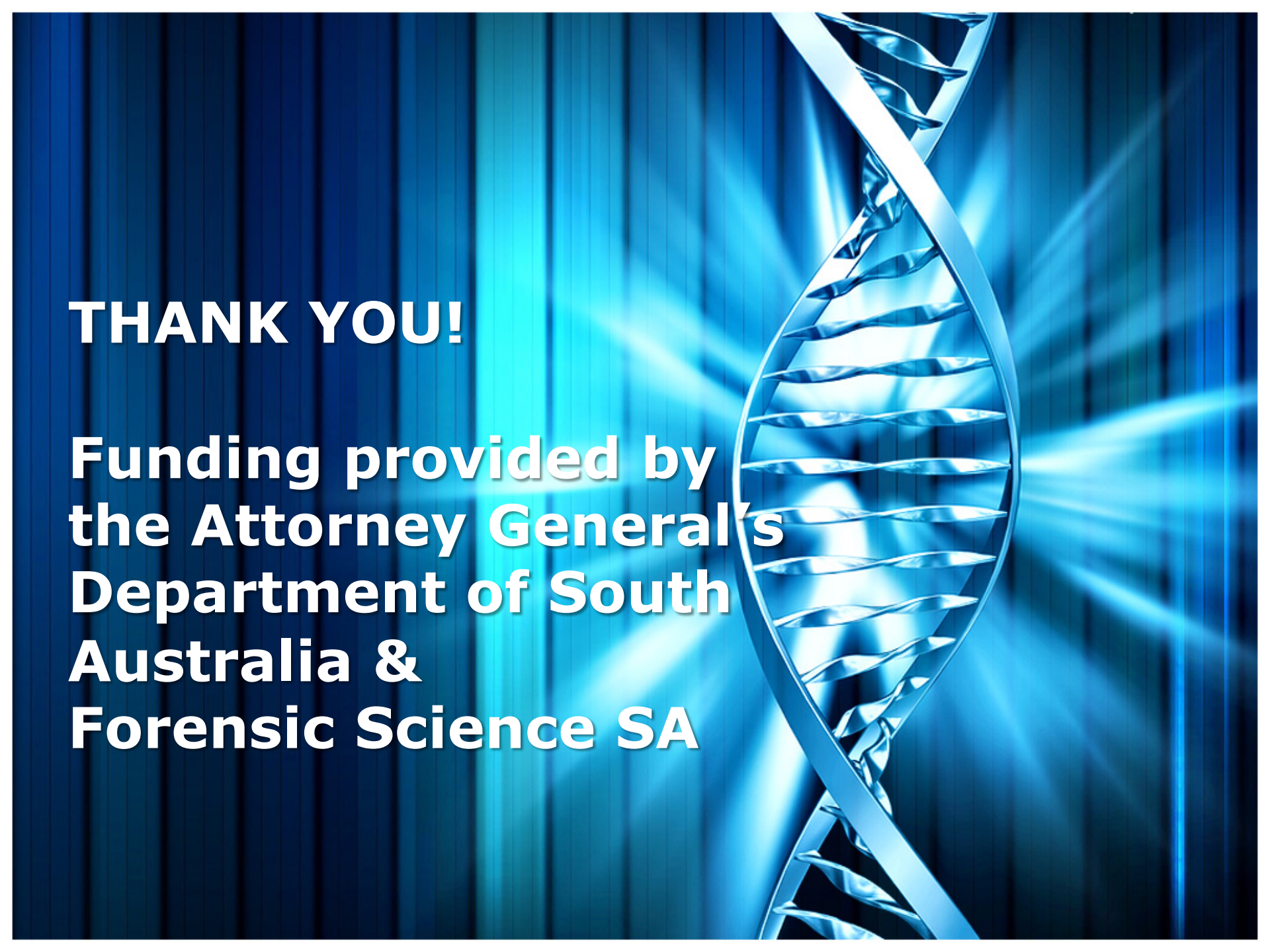
Zip-lock Bag



Zip-lock bag opened and then re-sealed. Inner fold targeted, swabbed, and direct PCR using Identifiler Plus. Full DNA profile matching the person opening the bag.

Concluding Comments

- Nucleic acid staining dyes can detect DNA deposited by touch
- The process is non-destructive, simple to perform and safe
- Latent DNA can be visualised with simple equipment
  - Can be performed in ambient light
- Allows the effective detection and collection of DNA
  - Targeted collection and monitoring real-time of transfer of DNA to swab


THANK YOU!
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