19TH WORLD STERILIZATION CONGRESS 2018

XIII INTERNATIONAL STERILIZATION CONGRESS AND HOSPITAL DISINFECTION

OCTOBER 31 TO NOVEMBER 3
2018 WORLD TRADE CENTER
MEXICO CITY

SCIENTIFIC PROGRAM

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Southampton
School of Biological Sciences

Cold Atmospheric Plasma to Decontaminate Surgical Instruments and Endoscopes

Bill Keevil PhD

Safe reuse of surgical instruments and endoscopes

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Efficient cleaning

Effective disinfection

Sterilisation, where feasible

Not feasible for heat-sensitive instruments such as endoscopes whilst cleaning and disinfection can be compromised by any instruments with lumens and/or complex geometries.

Cleaning and disinfection can be compromised by any instruments with lumens and/or complex geometries.

How can these procedures be monitored and validated?

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Infection control in clinical settings today

Steps in infection control	Tools available
Locate (and identify) the infectious agent(s)	Eyes, Detection kits, bioassays
Eliminate (or neutralize?) the infectious agent(s)	Equipment (AERs) and chemicals
Prevent introducing new infectious agent(s)	SOPs (based on RA)

1. How Dead is Dead following "Cleaning"?

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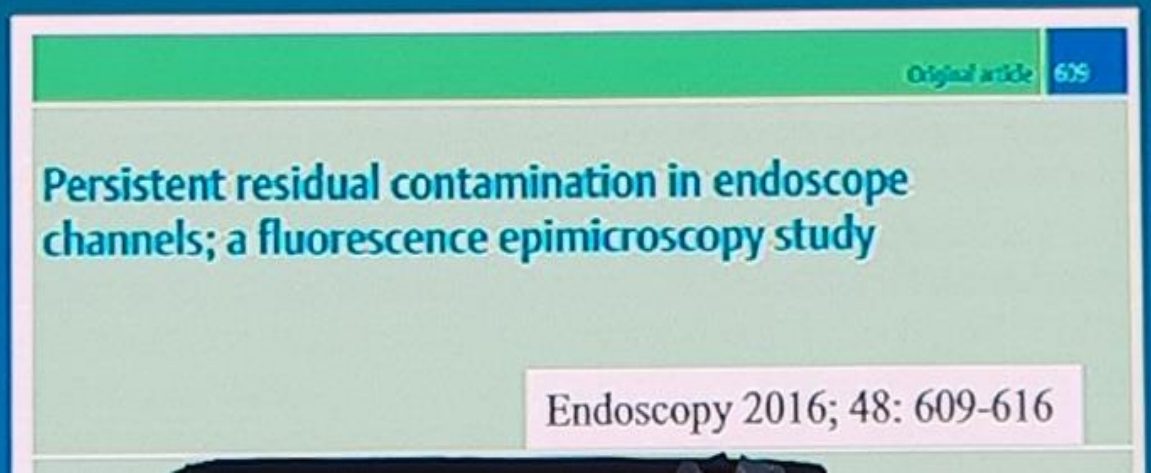
Recent outbreaks of biofilm-related antibiotic resistant strains such as *Klebsiella pneumonia* associated with complex:

- duodenoscopes
- gastroscopes
- cystoscopes
- uretoscopes



Channels recovered from endoscopes in clinical use: BIOFILM

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Brush marks show biofilm not removed during reprocessing

Poor drying of luminal endoscopes

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Residual water micro-droplets inside working channel after cleaning and passage of forced air

evil / JHI 83 (2013) 22-29

American Journal of Infection Control 42 (2014) 1283-6

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

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Major article

Correlation between the growth of bacterial biofilm in flexible endoscopes and endoscope reprocessing methods

Wu Ren-Pei MD [a,1], Xi Hui-Jun MD [a,1], Qi Ke MD [a], Wang Dong MD [a], Nie Xing PhD [b], Li Zhao-Shen PhD, MD [a,*]

American Journal of Infection Control 42 (2014) 1283-6

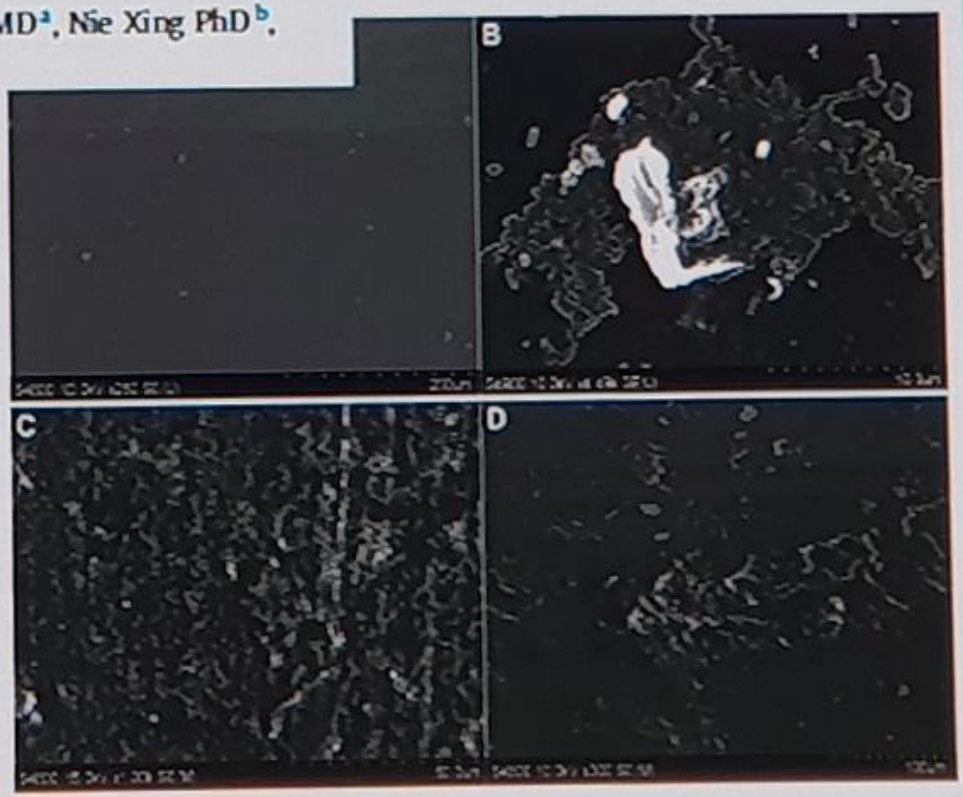

Fig 1. Biofilm growth on the inner surface of suction and biopsy channels of endoscopes used clinically.

practice may be related to

2. How Clean is Clean?

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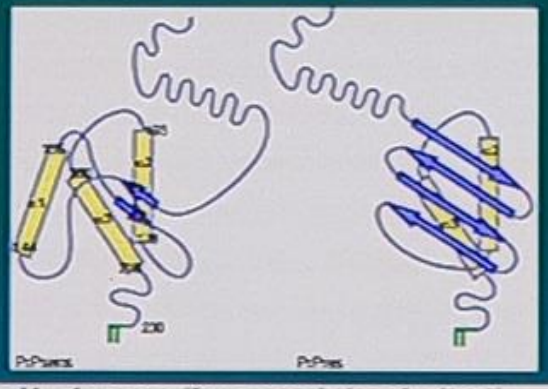
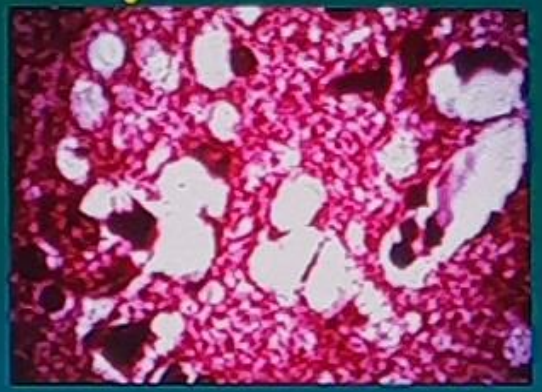
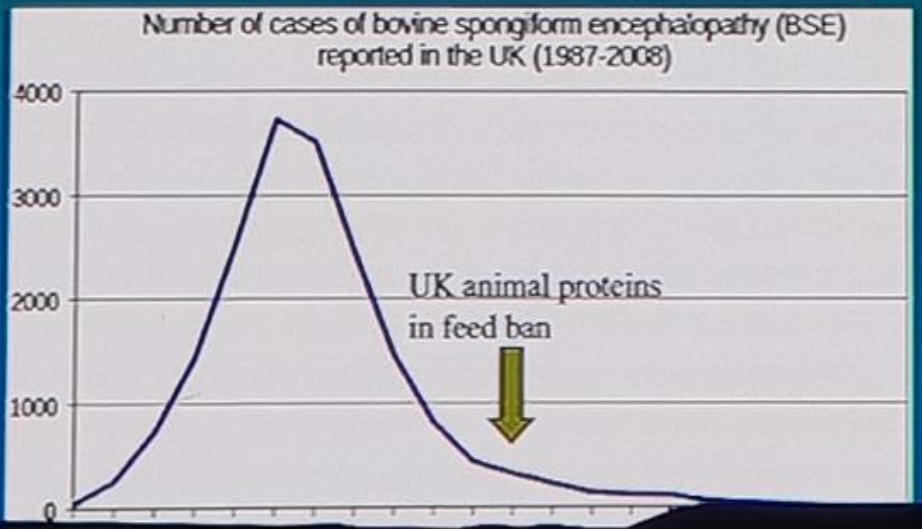
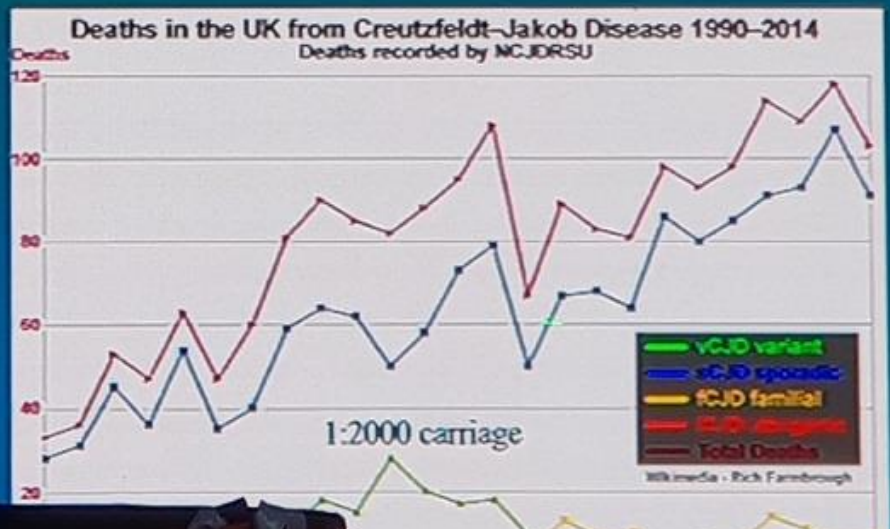
Current monitoring of protein residues is insensitive
Still concerned with infectious vCJD amyloid prion contamination post-surgery. Bind strongly, resistant to temp, alkali/ enzyme cleaners









Spaulding classification

Earle H. Spaulding "strategy for sterilization or disinfection of inanimate objects and surfaces based on the degree of risk involved in their use" (1939)

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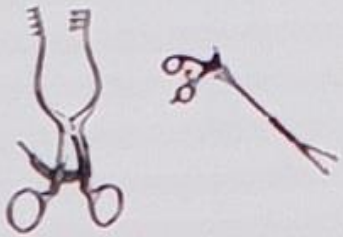
Patient Contact	Examples	Device Classification	Minimum Inactivation Level
Intact skin		Non-Critical	Cleaning and/or Low/Intermediate Level Disinfection
Mucous membranes or non-intact skin		Semi-Critical	High Level Disinfection *
Sterile areas of the body, including blood contact		Critical	Sterilization Prions?

*

3. How Safe is Safe?

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New recommendations: protein contamination on surgical instruments should be < 3 or 5 ug protein per instrument side

English Dept of Health HTM01-01Guidance requires SSDs "should no longer rely on elution or swabbing to detect residual protein on an instrument.

The method should be validated as being able to detect protein equivalent to ≤5 µg of BSA *in situ* on the surface of an instrument. Commercial technologies that can detect the 5 µg limit *in situ* are being developed (see ACDP- TSE's Annex C).

Methods that do not have protein as their target, such as ATP assays, cannot be used as a substitute for residual protein detection.

Insensitive Biuret Test

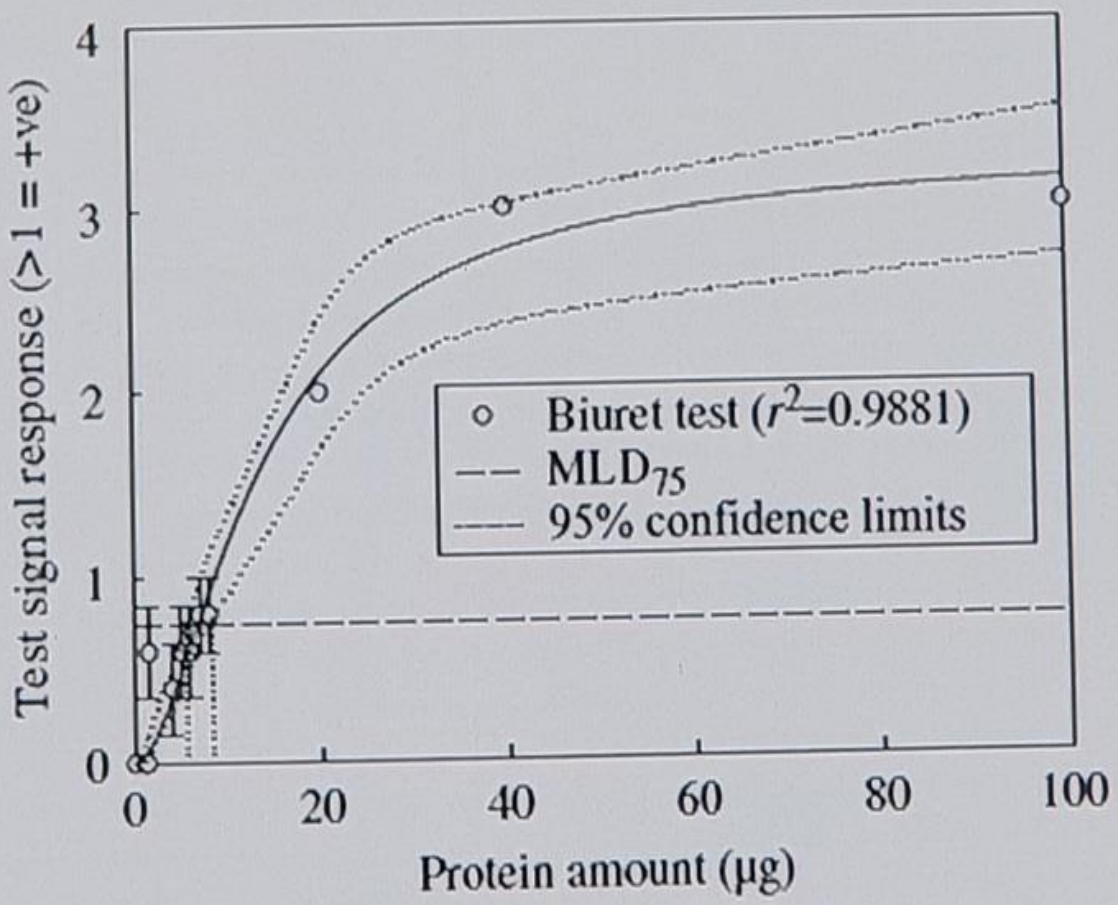
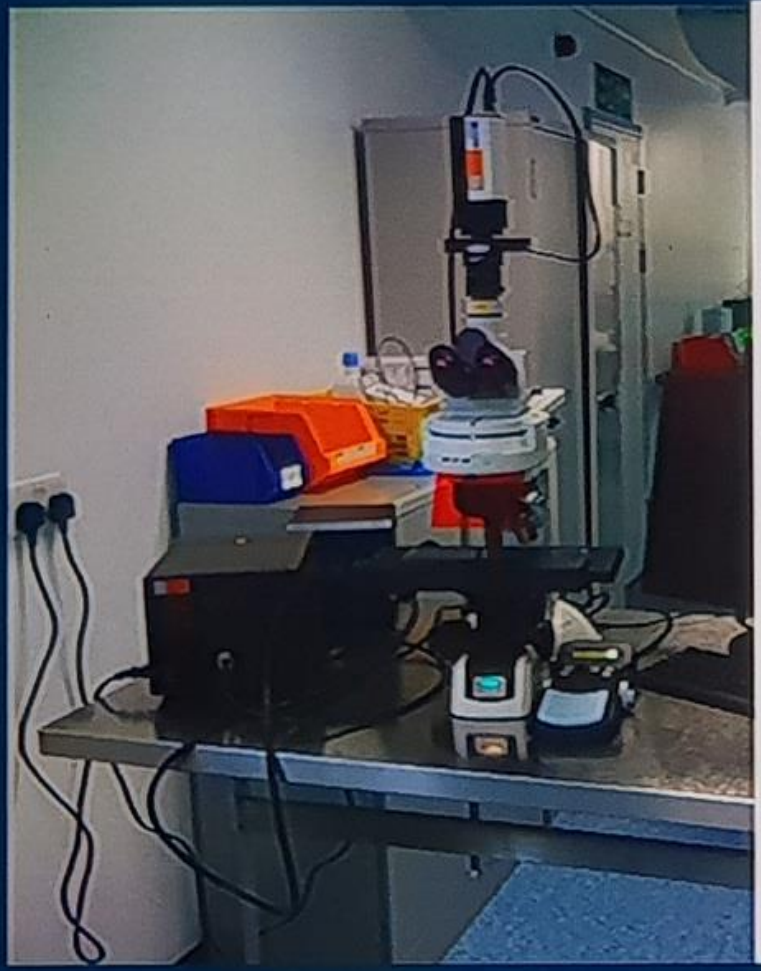
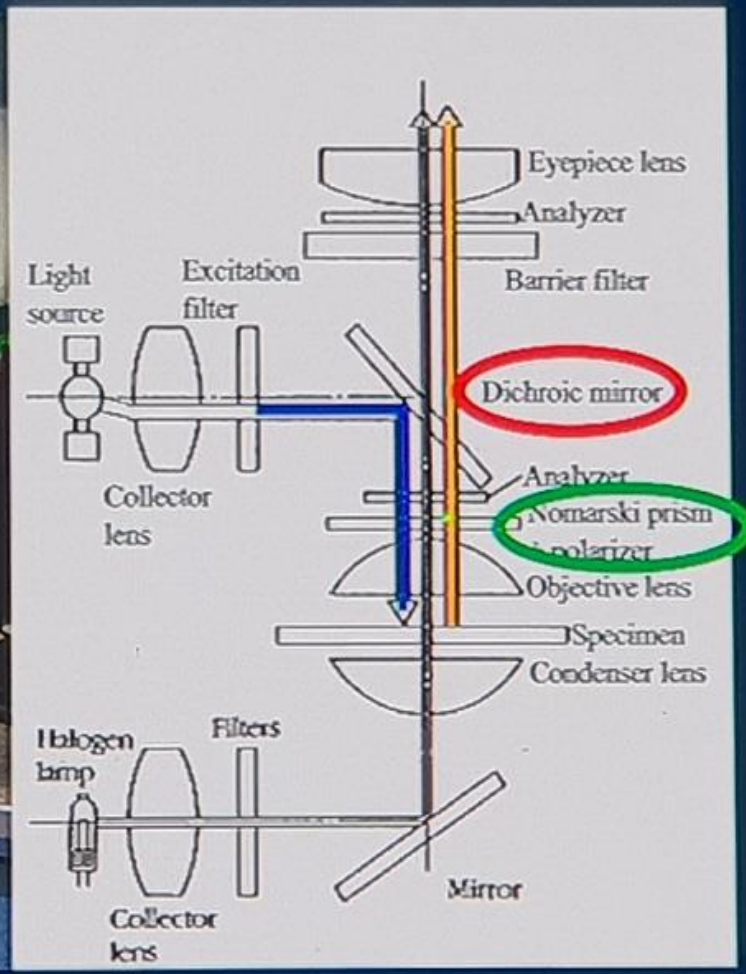


Figure 3 Biuret test sensitivity results giving a minimum level of detection observed by 75% of volunteers (MLD_{75}) sensitivity level of 6.7 µg (95% confidence interval 5.4–8.2 µg).

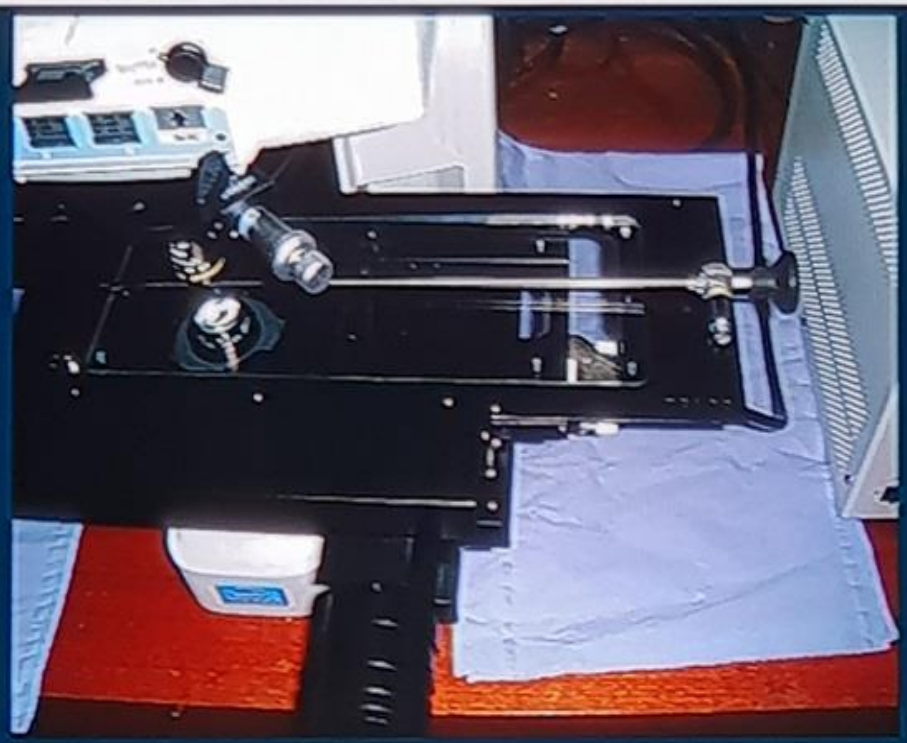


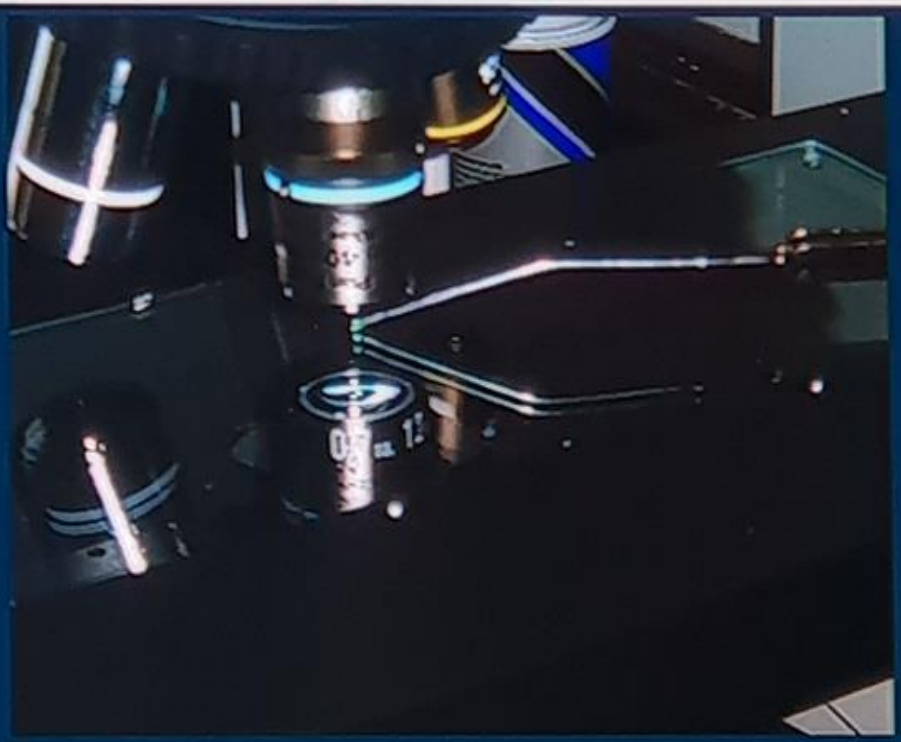


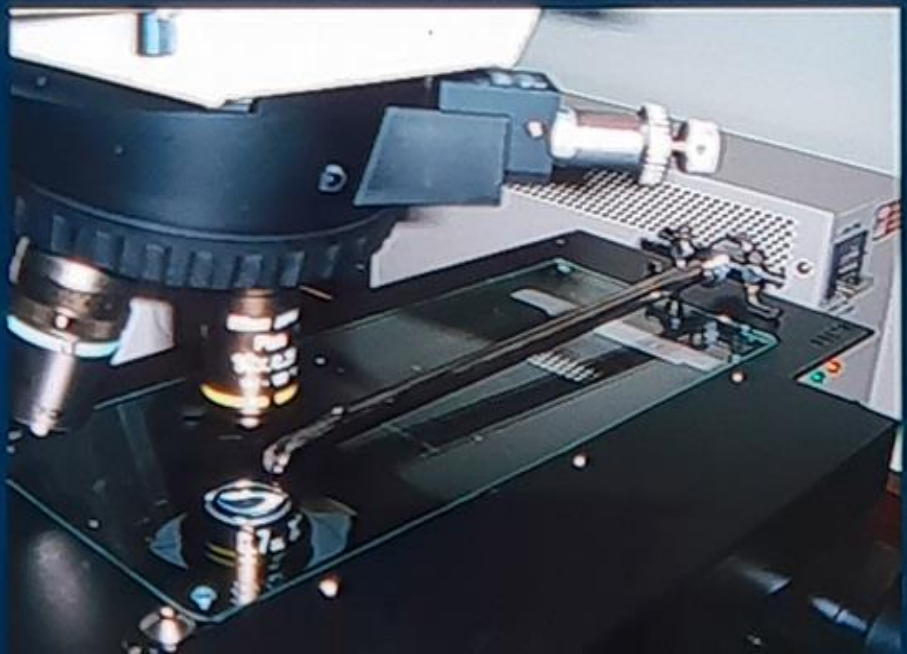
Episcopic Differential Interference Contrast with Epifluorescence (EDIC/EF) microscopy.

Keevil *et al.*, Water Sci Technol 2003

Linscomb *et al.*, IHI 2006

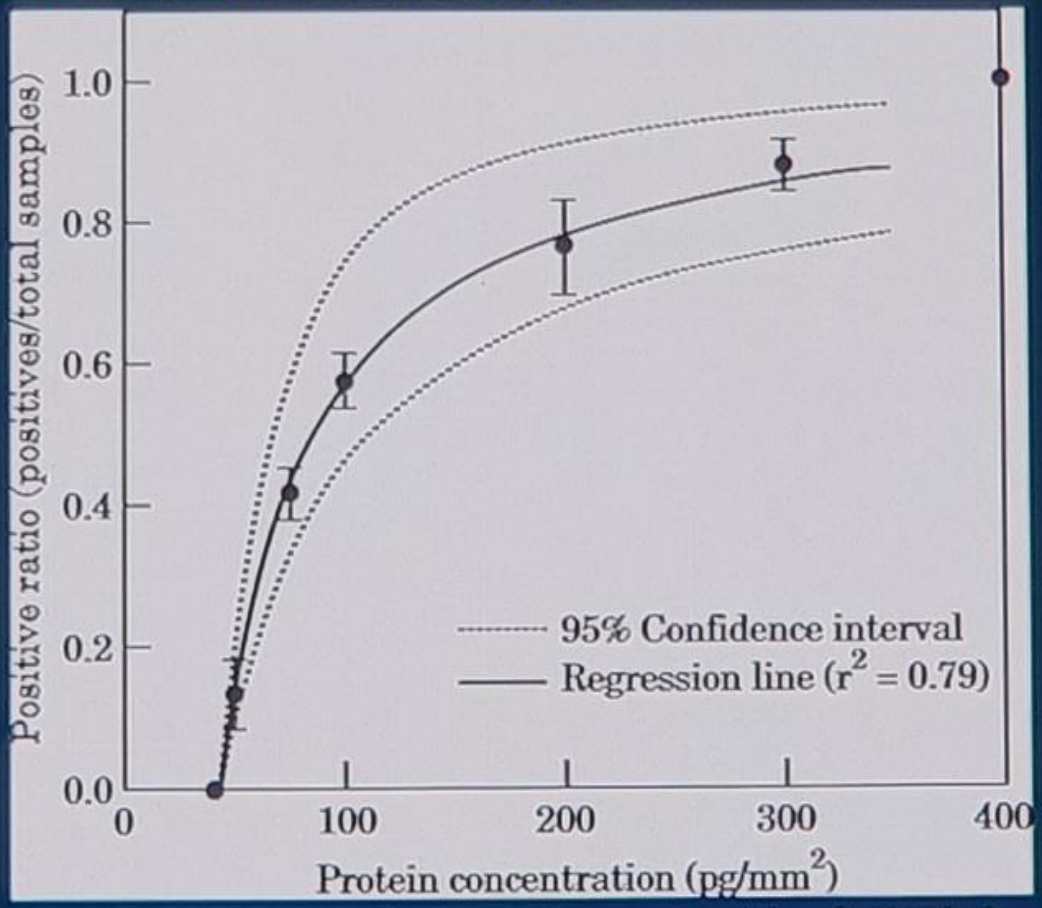






Unique features of the EDIC/EF microscope enable a range of medical instruments to be inspected

SYPRO Ruby Sensitivity of Protein Detection

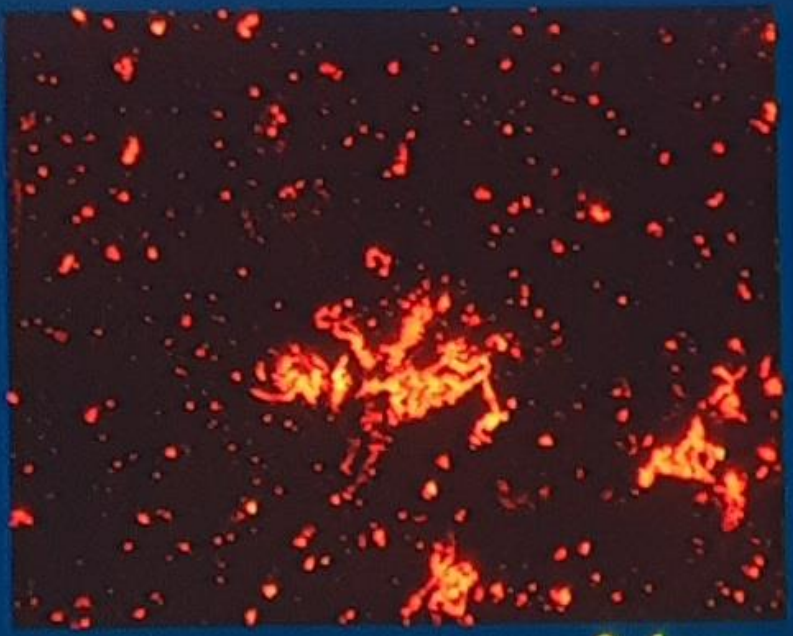


Results of dilution series showing where results were positive for brain homogenate (N=8 observers, 12 replicates per observer) on stainless steel surfaces. The minimum level of detection observed by 50% of volunteers was 85 pg/mm^2 (95% confidence intervals 67–112 pg/mm2). Lipscomb et al., JHI 2006.

- MLD_{75} = 175 pg/mm^2 (95% CI 104 – 286 pg/mm^2)

COMPARISON WITH BIURET AND NINHYDRIN TESTS

Protein concentrations assessed by direct EDIC/EF microscopy



0.4 µg

Clean surgical instruments observed under EDIC/EF

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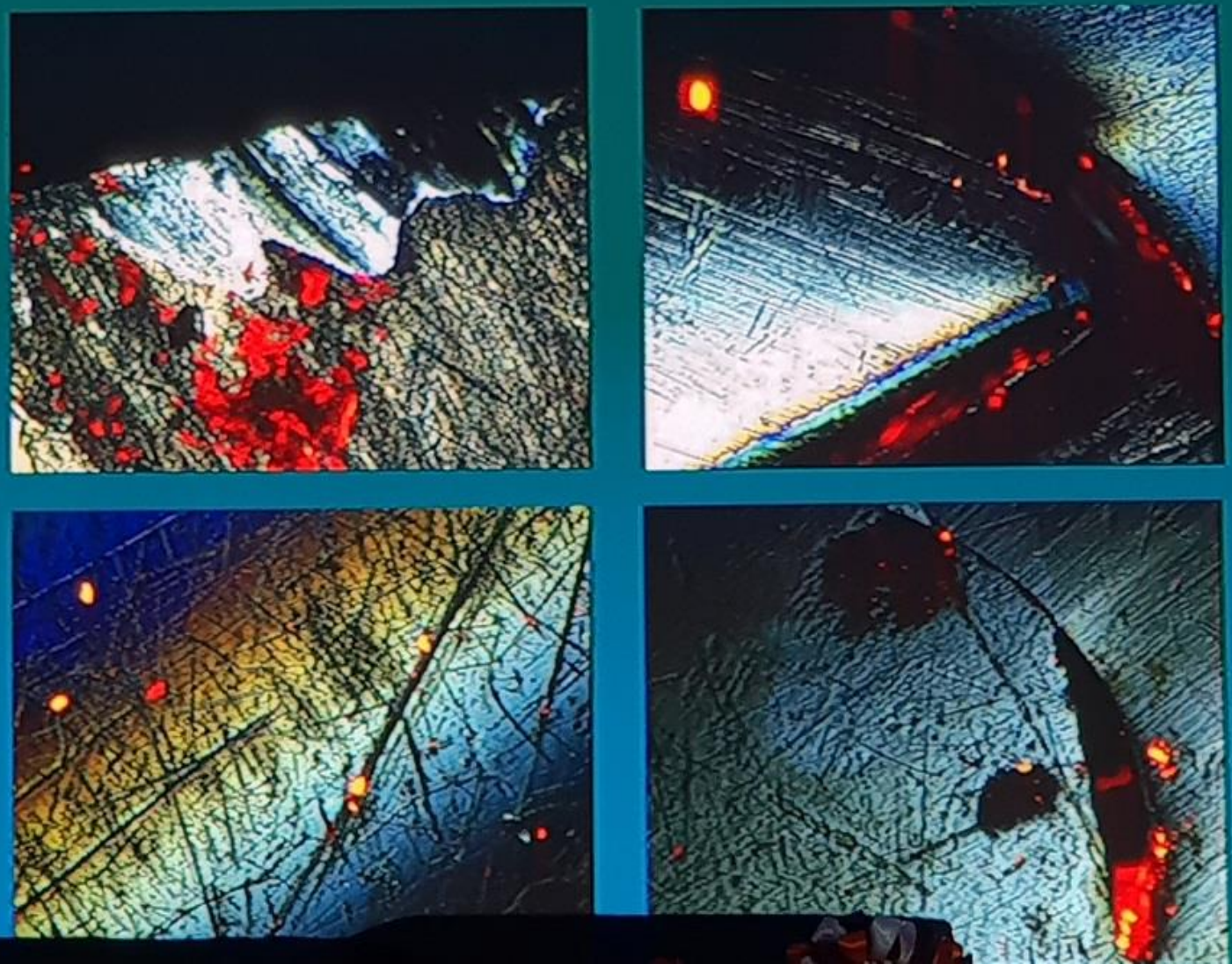
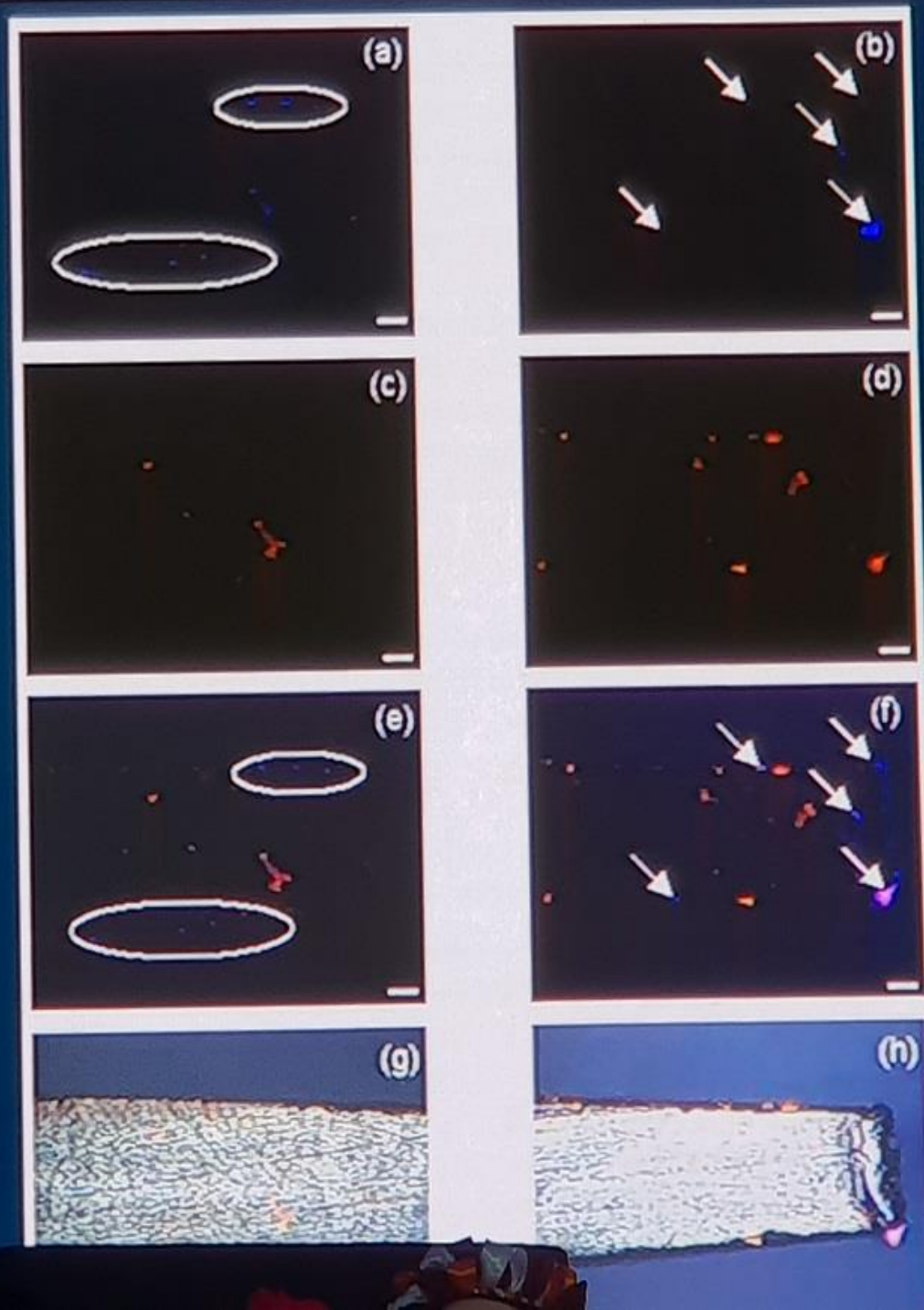


EDIC/EF microscopy of ME7-infected brain homogenate contamination on 2 different areas of surgical forceps

Bars: 100 µm

Approx 47 µg of tissue protein and 640 pg of PrP^{Sc} amyloid were detected per mm^2 of the instrument surface after only **one second contact time** - equates to **18 femtomoles of PrP^{Sc} per mm^2**

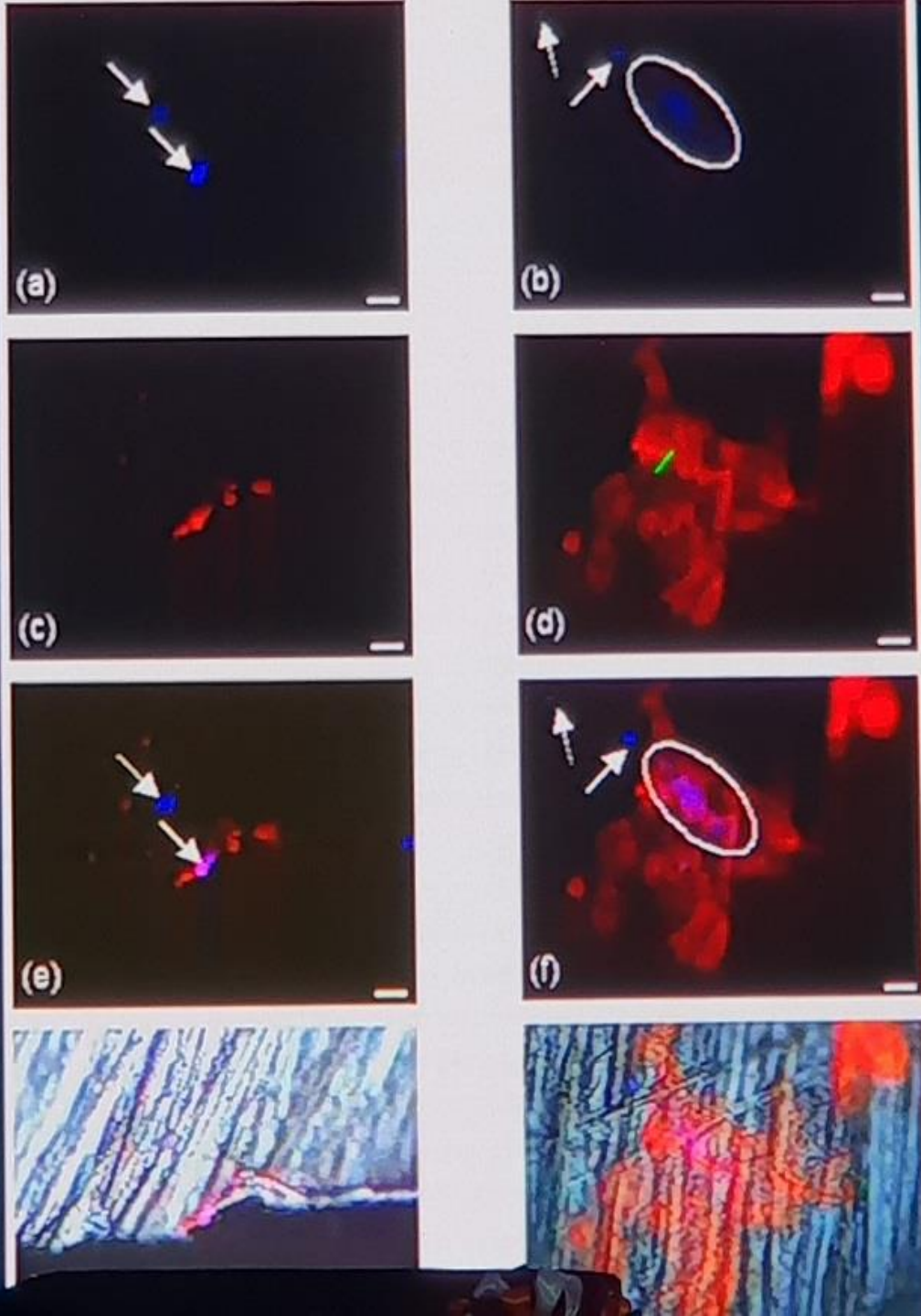


High magnification EDIC/EF microscopy of ME7-infected brain homogenate contamination on 2 different areas of surgical forceps

Bars: 10 µm

Tissue proteins do not tend to aggregate around amyloid cores.

Contamination tended to aggregate in grooves or pits.



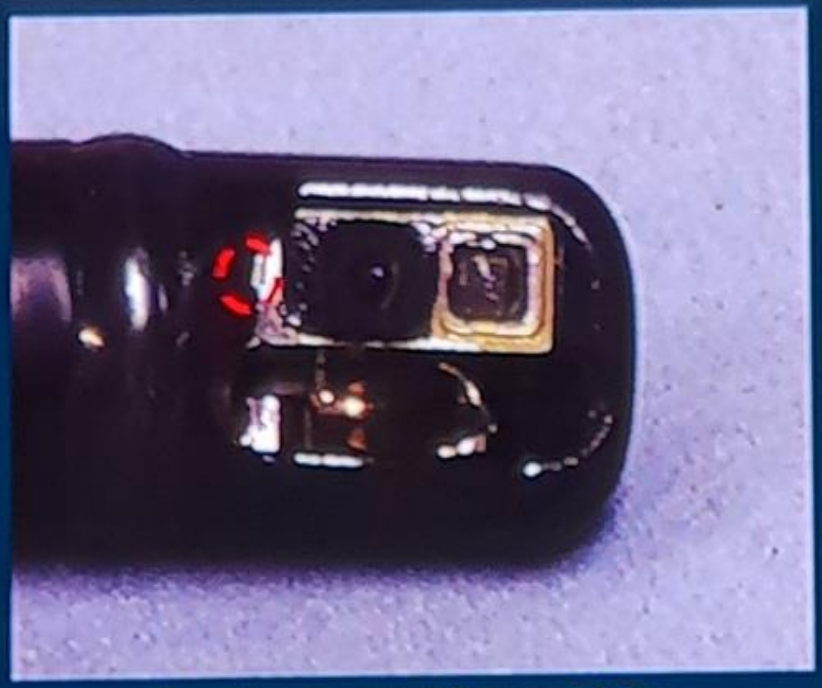
ThT

SR

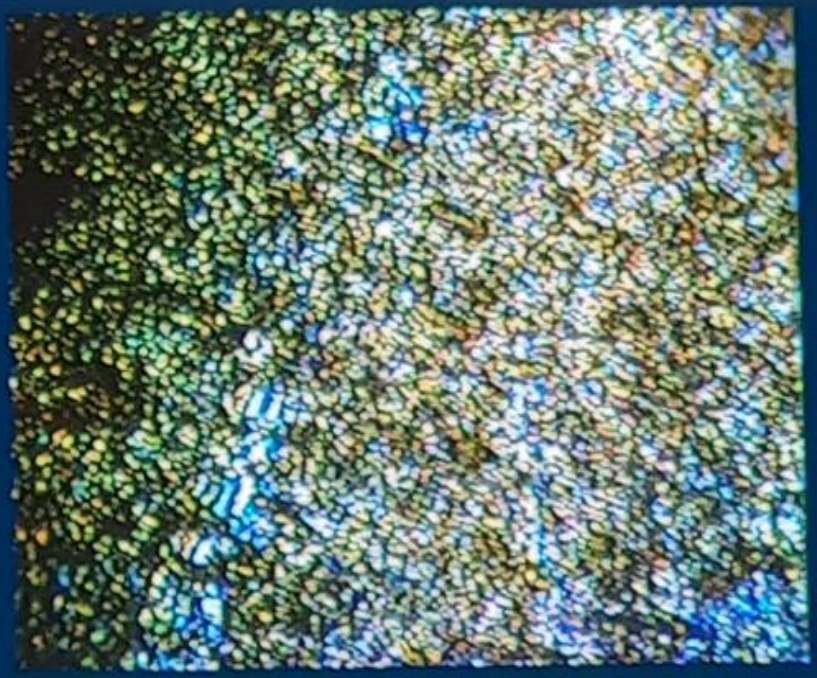
ThT/SR

EDIC/ ThT/SR

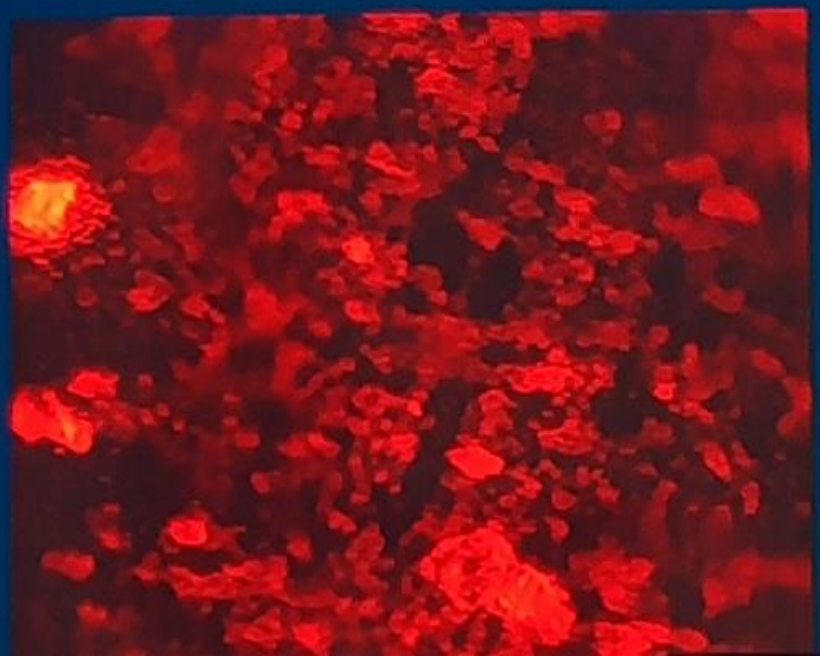
Flexible Endoscope Studies:

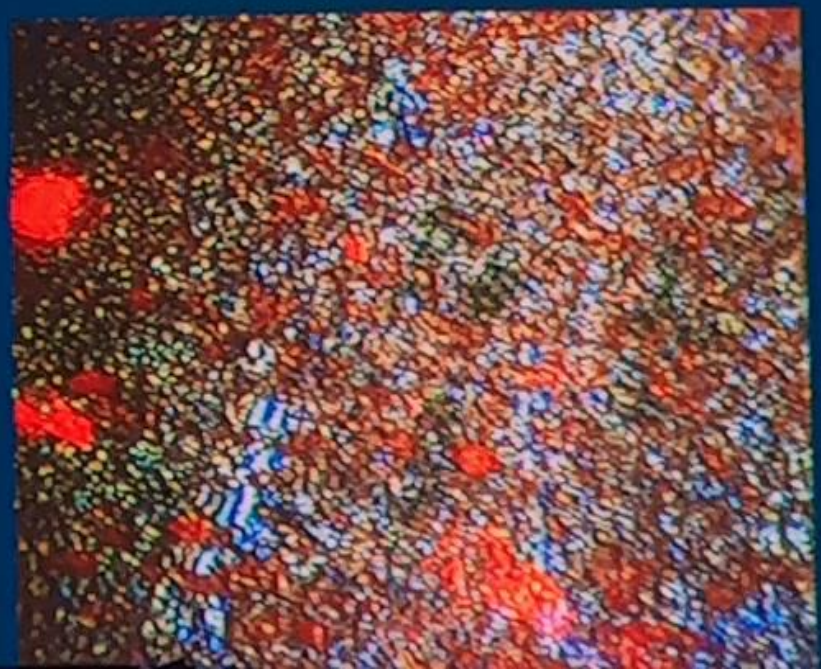


Duodenoscope stainless steel ledge



Proteinaceous deposits on SS ledge (x 600)





Luminal endoscopes

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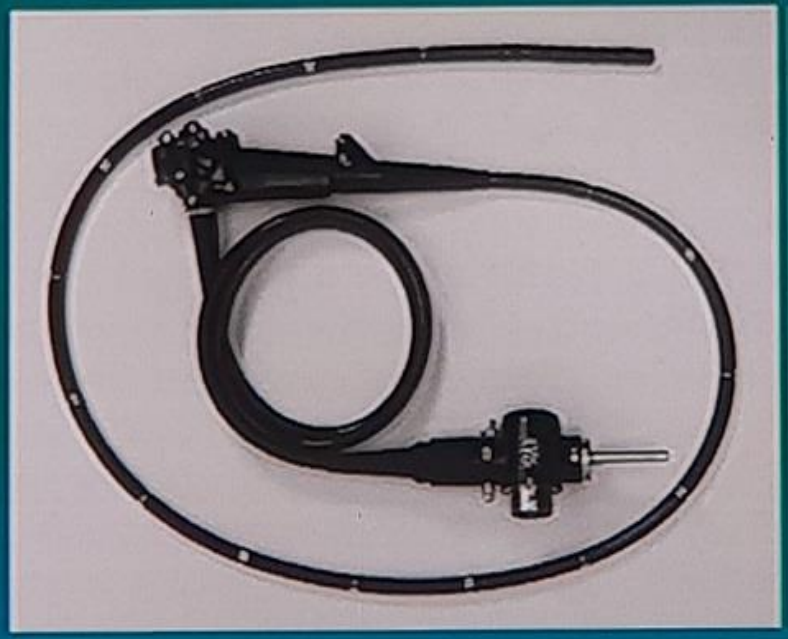

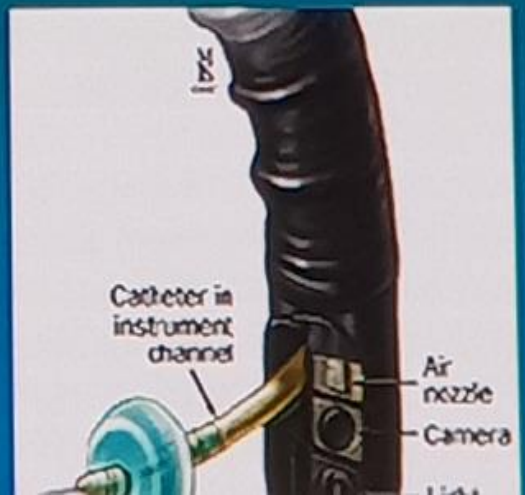

Reprocessing luminal flexible endoscopes

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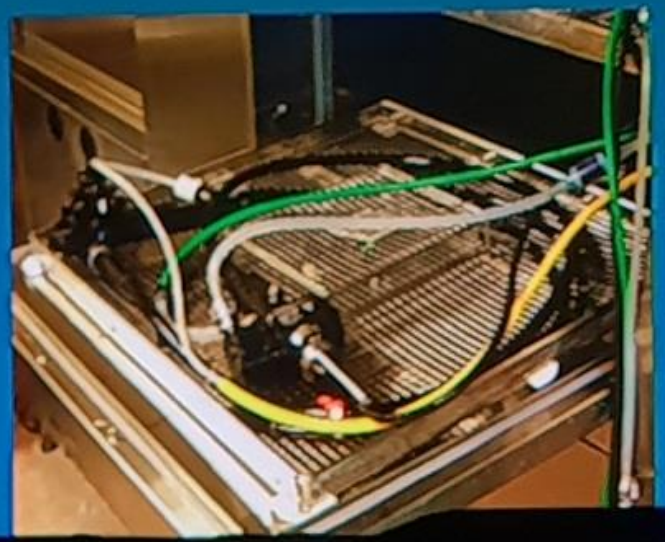

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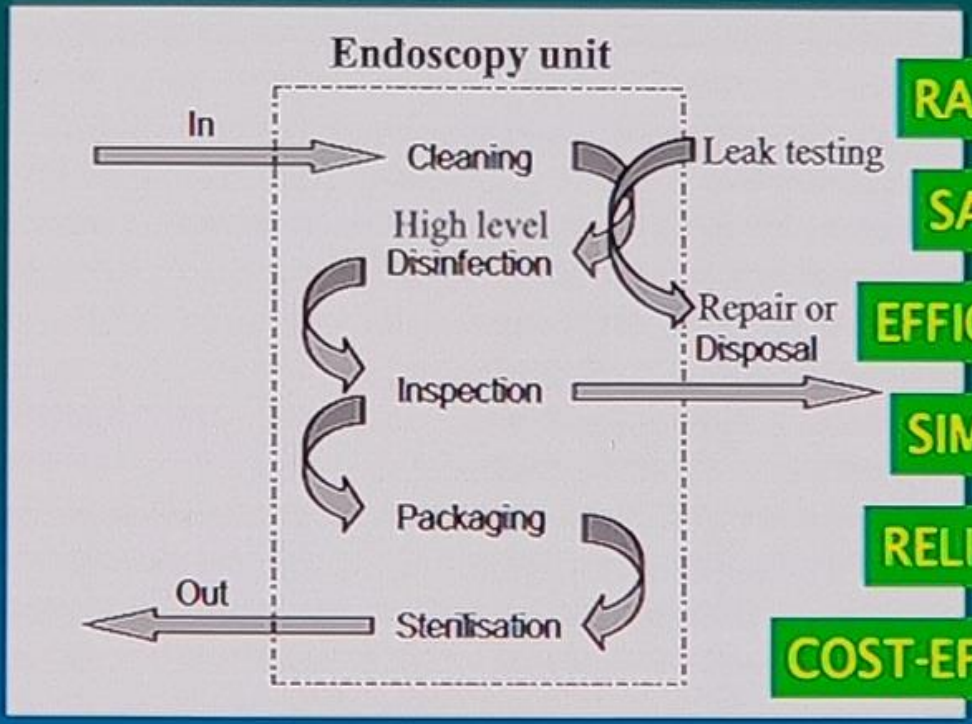
Reducing the risks of iatrogenic infection through endoscopy



The five main functions performed in a hospital sterile service department

and the practical requirements

Reducing the risks of iatrogenic infection through endoscopy

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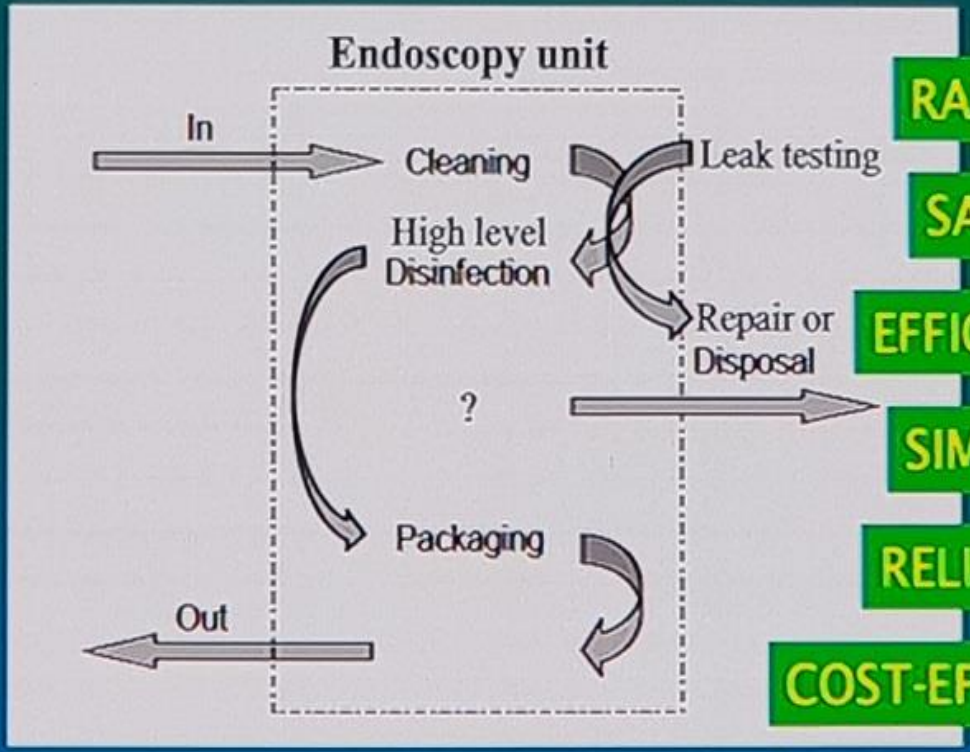

The five main functions performed in a hospital sterile service department

and the practical requirements

Channels recovered from endoscopes in clinical use

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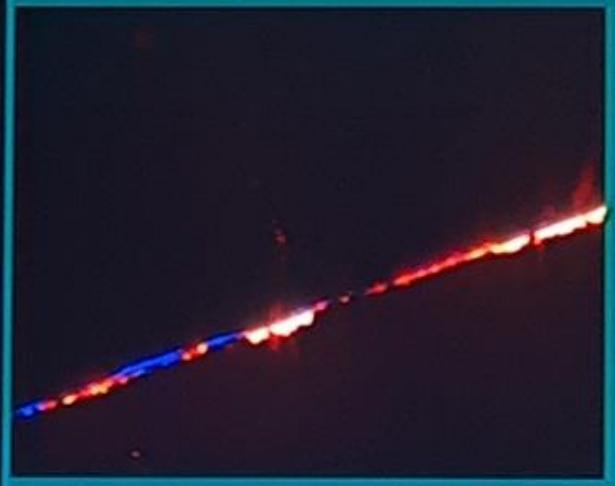

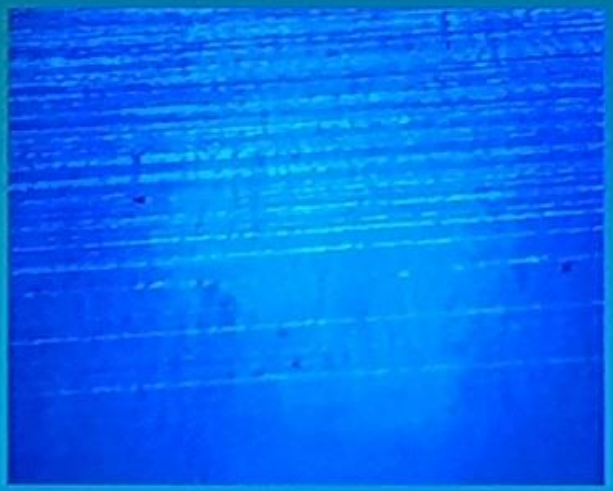

gastroscope

colonoscope

bronchoscope

100µm

Channels recovered from endoscopes in clinical use

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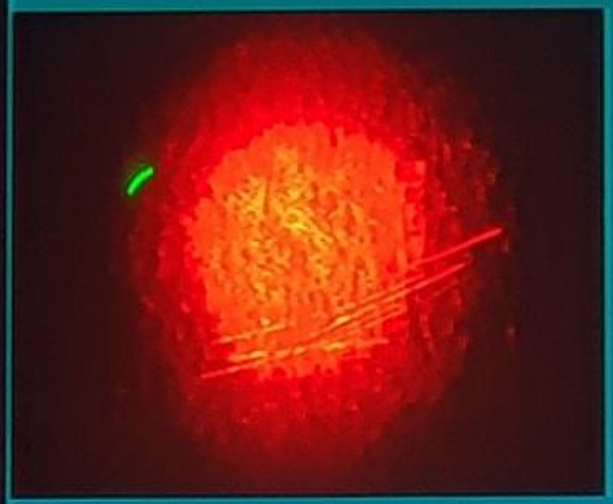

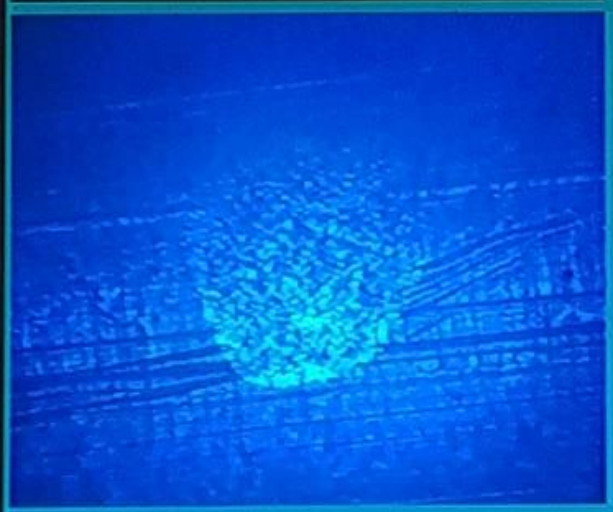

colonoscope

colonoscope

bronchoscope

100µm

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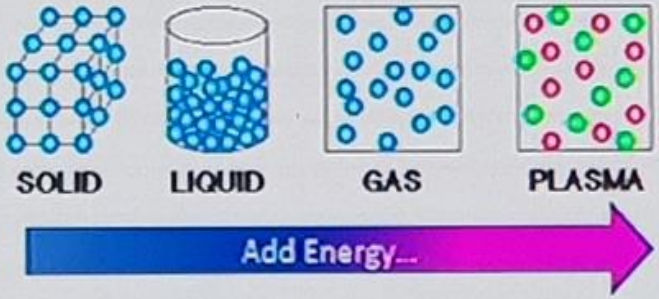
Plasma – the 4th state of the matter



The forth state of matter was first 'discovered' by Sir William Crookes in 1879.

-It was first described as 'plasma' by Irving Langmuir in 1928.

plasma " 'region containing balanced charges of ions and electrons"

blood plasma "variety of different 'corpuscles' suspended in liquid"

Plasma - a state of matter similar to gas in which a certain portion of the particles are ionized. categorized into **cold** and **hot** plasmas based on an electron temperature / ionization rate.

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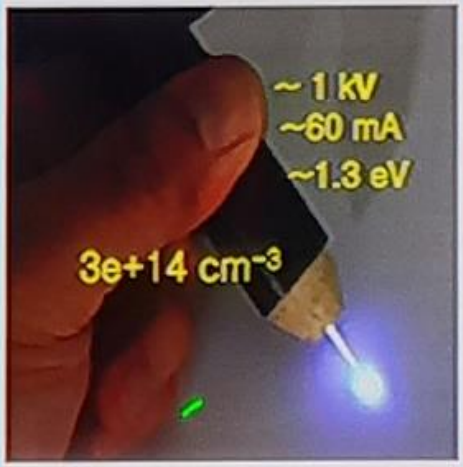
- **Plasma Discharges**

	Corona Discharge (CD)	Dielectric barrier Discharge (DBD)	Atmospheric Pressure Plasma Jet (APPJ)	Micro Hollow Cathode Discharge (MHCD)
Electrode Configuration	Sharply pointed electrode	Electrodes are covered by dielectric materials		Micro Hollow cathode
Ignition method	Pulsed DC	Pulsed DC / AC / RF	Pulsed DC / RF	DC
Operating pressure	Atmospheric pressure	Atmospheric pressure	Atmospheric pressure	Atmospheric pressure
Breakdown Voltage	10 ~ 50 kV	1 ~ 10 kV	0.05 ~ 2 kV	
Operating gas temperature	300 K	300 K	400 K	2000 K
Shield gas requirement	No	No	Yes	Yes
Scalability	No	Yes	Yes	Yes

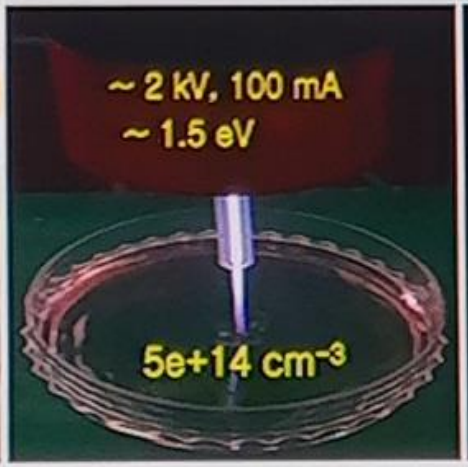
Plasma devices for biomedical applications

Under Air Plasma

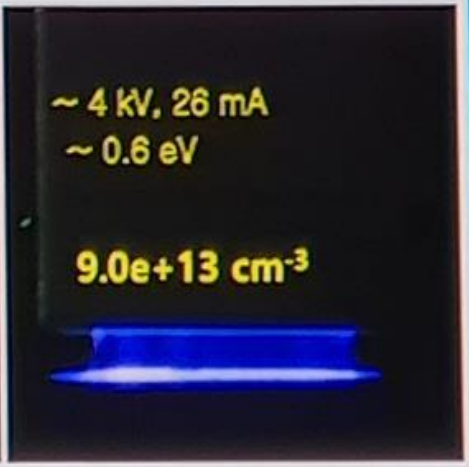


Soft Jet plasma

Microwave Jet plasma

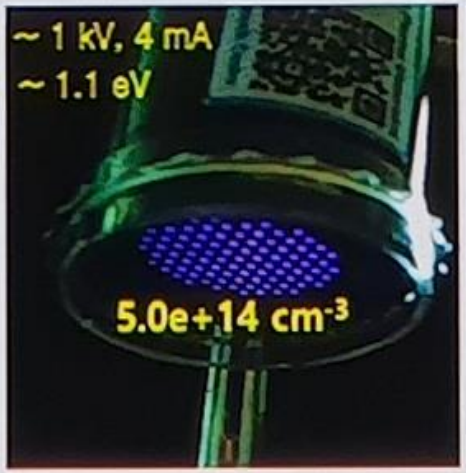


FE-DBD Jet plasma



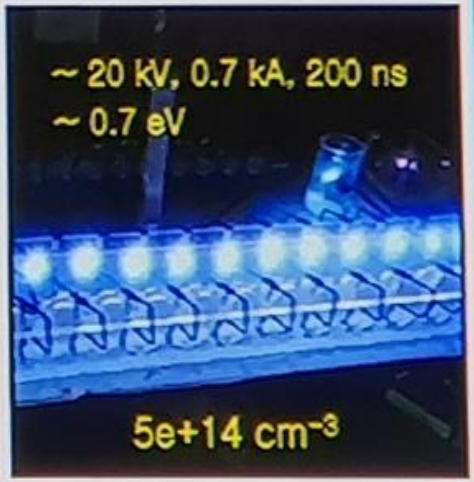
FE-DBD plasma

μ-DBD plasma (90mm)



μ-DBD plasma (35mm)

Annular Jet plasma



Nanosecond pulse plasma

CAP endoDecon | Versatility and Uniformity Control

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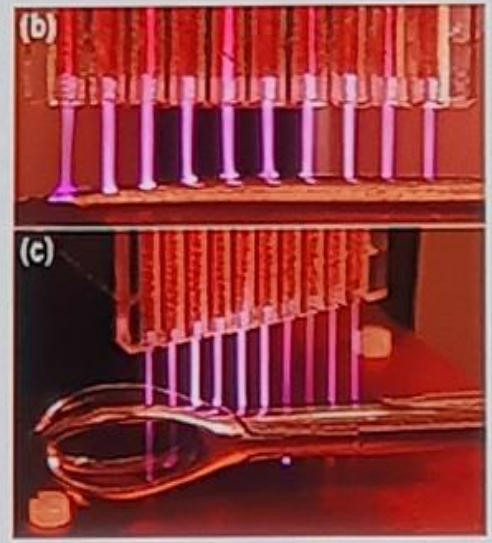
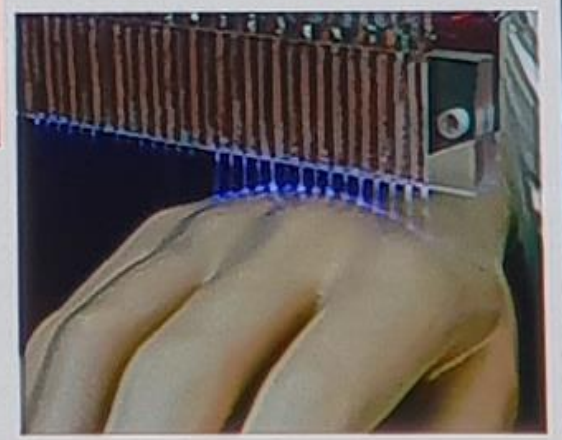


The long lumen challenge



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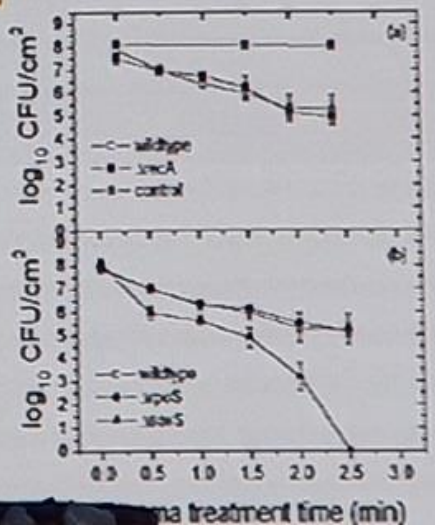
Biological applications – Decontamination – How?

Synergy

- OH
- O
- O_2^-
- NO
- H_2O_2
- O_3
- UV photons
- E – Fields

E. coli

2. Live/Dead stain



1. Culture recovery

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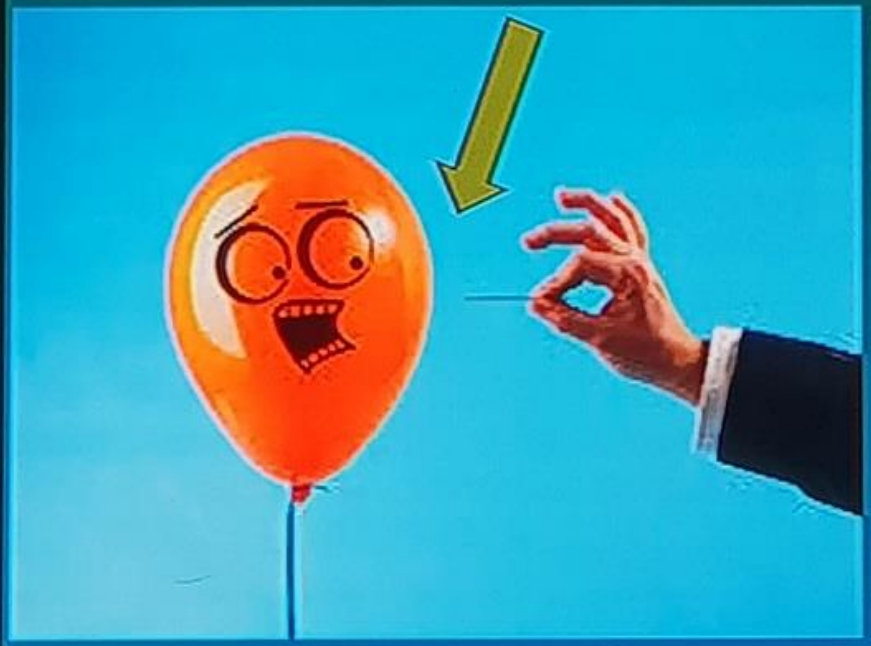
How Dead is Dead?

Plasma ion



How Clean is Clean?

Dead but not Gone –
LPS Endotoxin; Prions?

PNG

Shutterstock.com

Endoscope channels: residual protein after combined CAP/ enzymatic cleaning against Browne soil.

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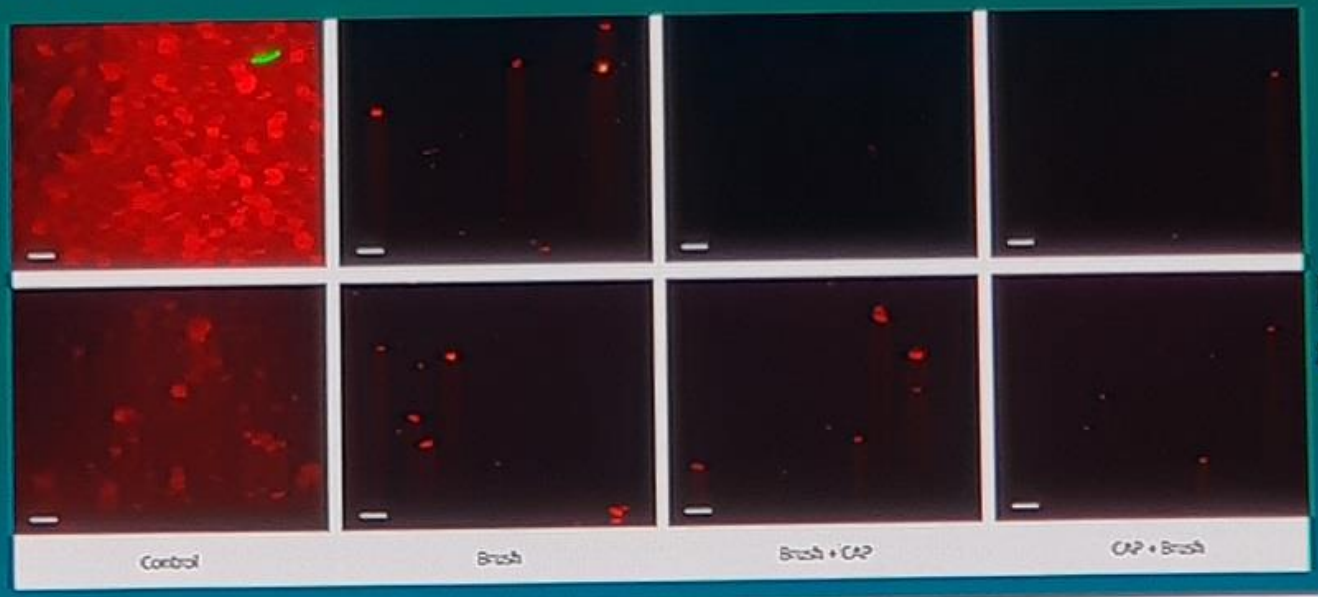
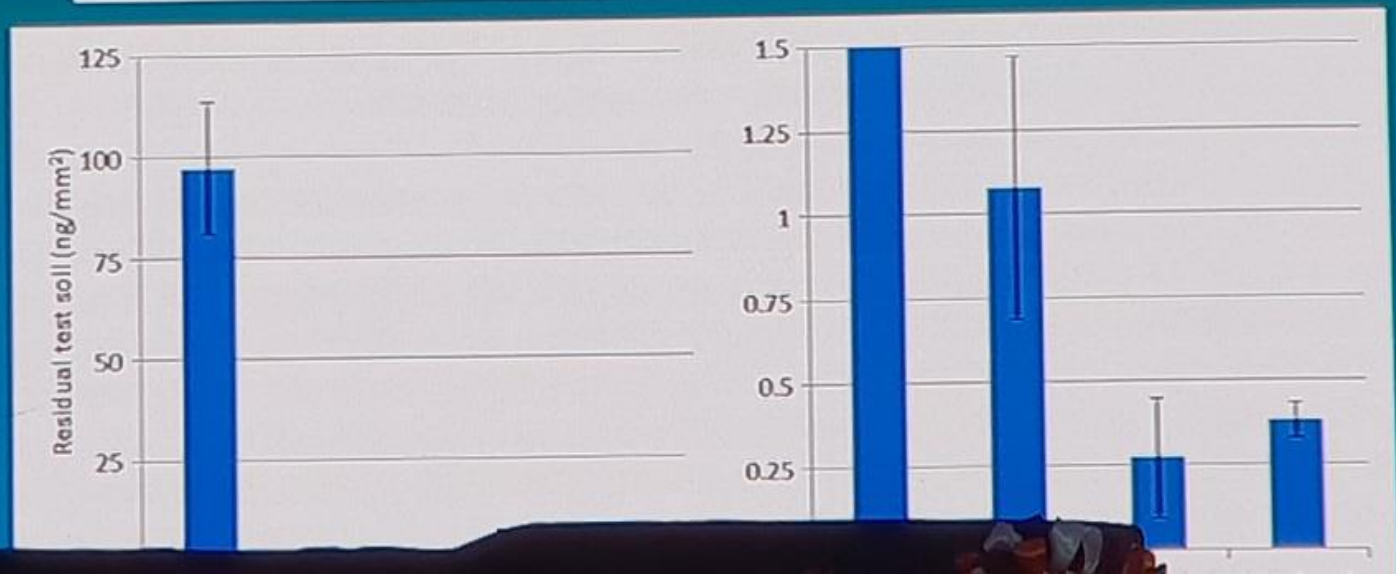


Micrograph bars are 100 µm



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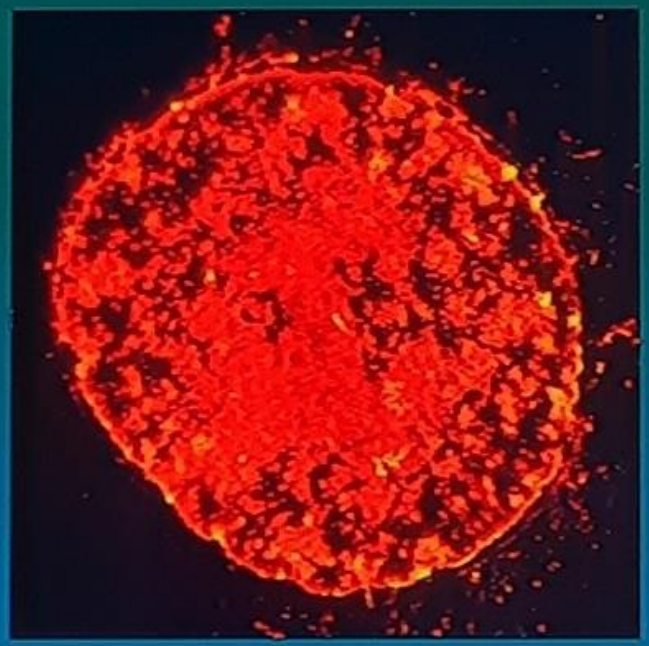
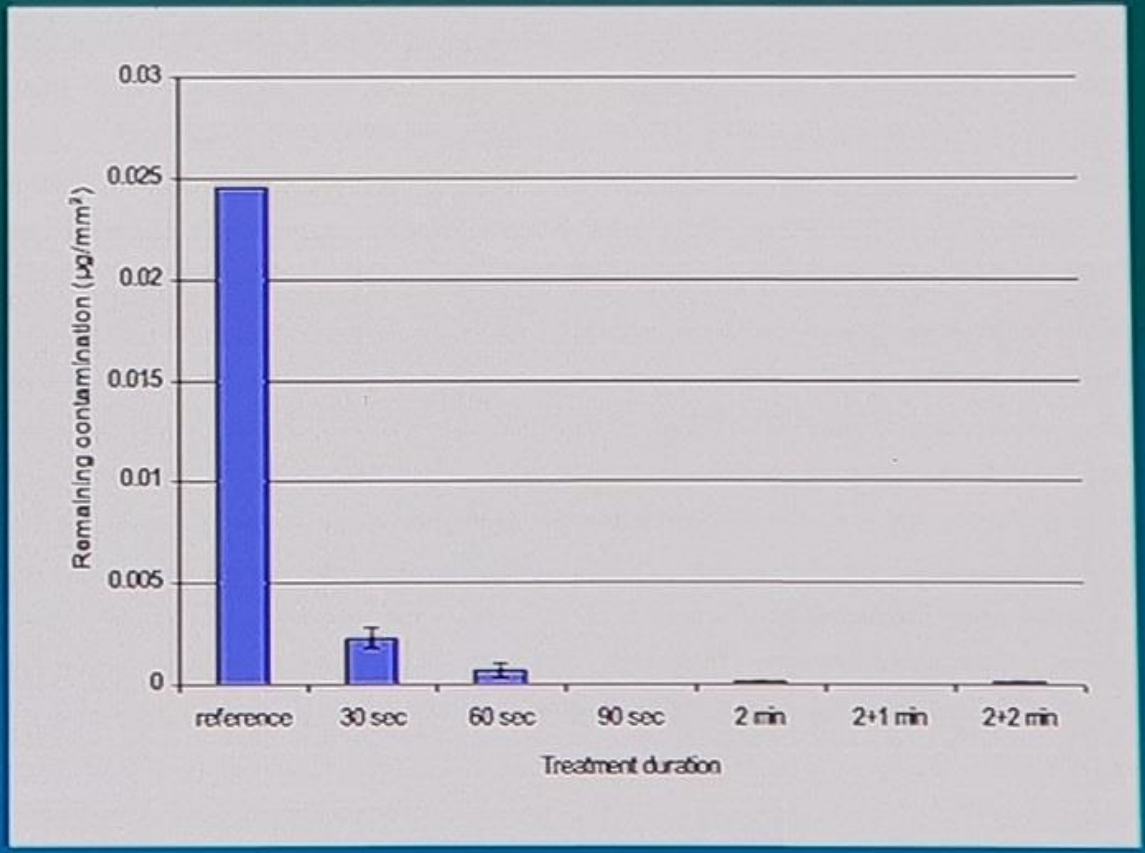
Surgical stainless steel

CAP prototype: He/O_2 gas



Control mouse brain homogenate
(protein content: 1µg)



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Animal infectivity assays:

Wire implant in brain model

363K Scrapie
Spiked implant wires before CAP treatment

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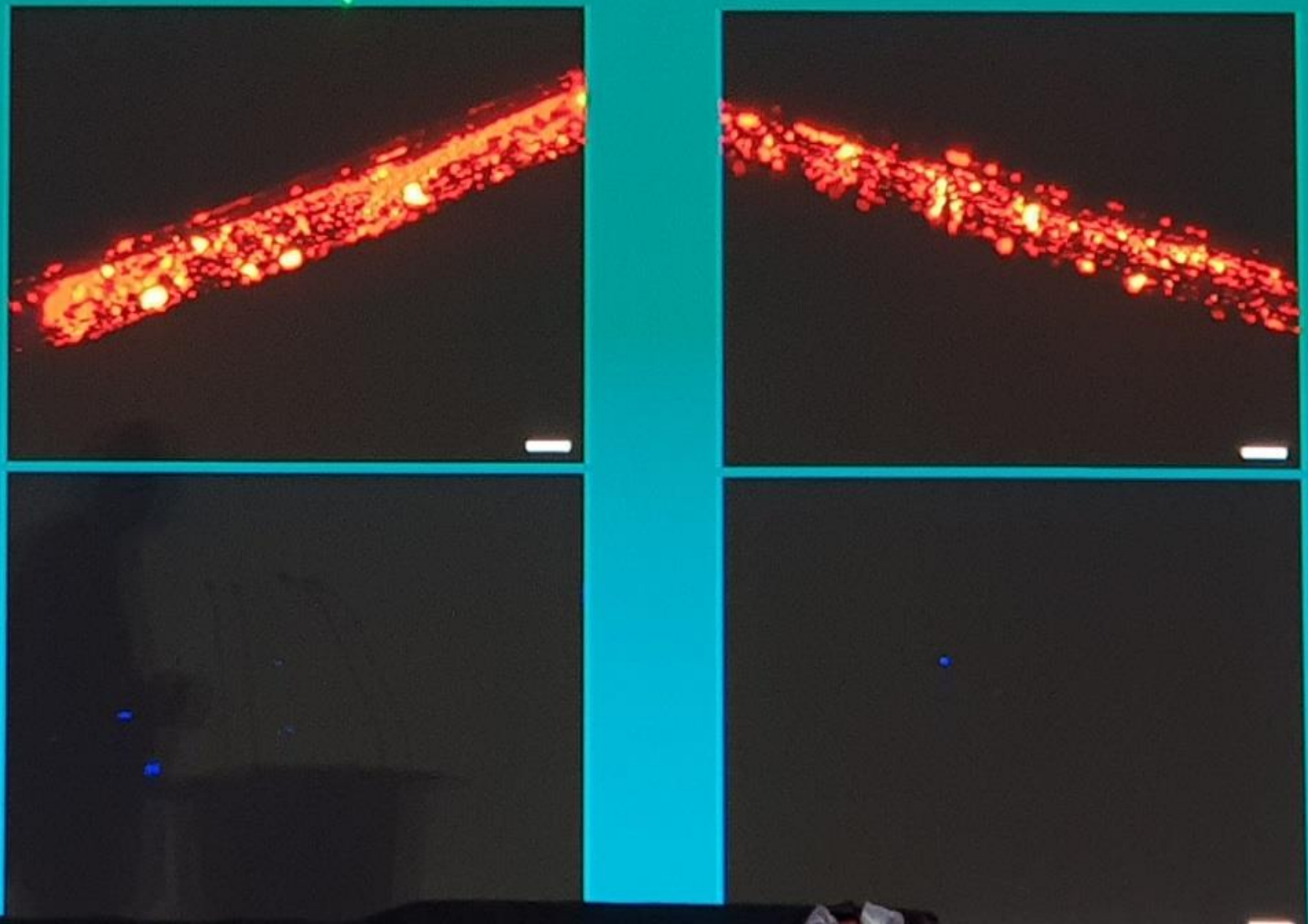

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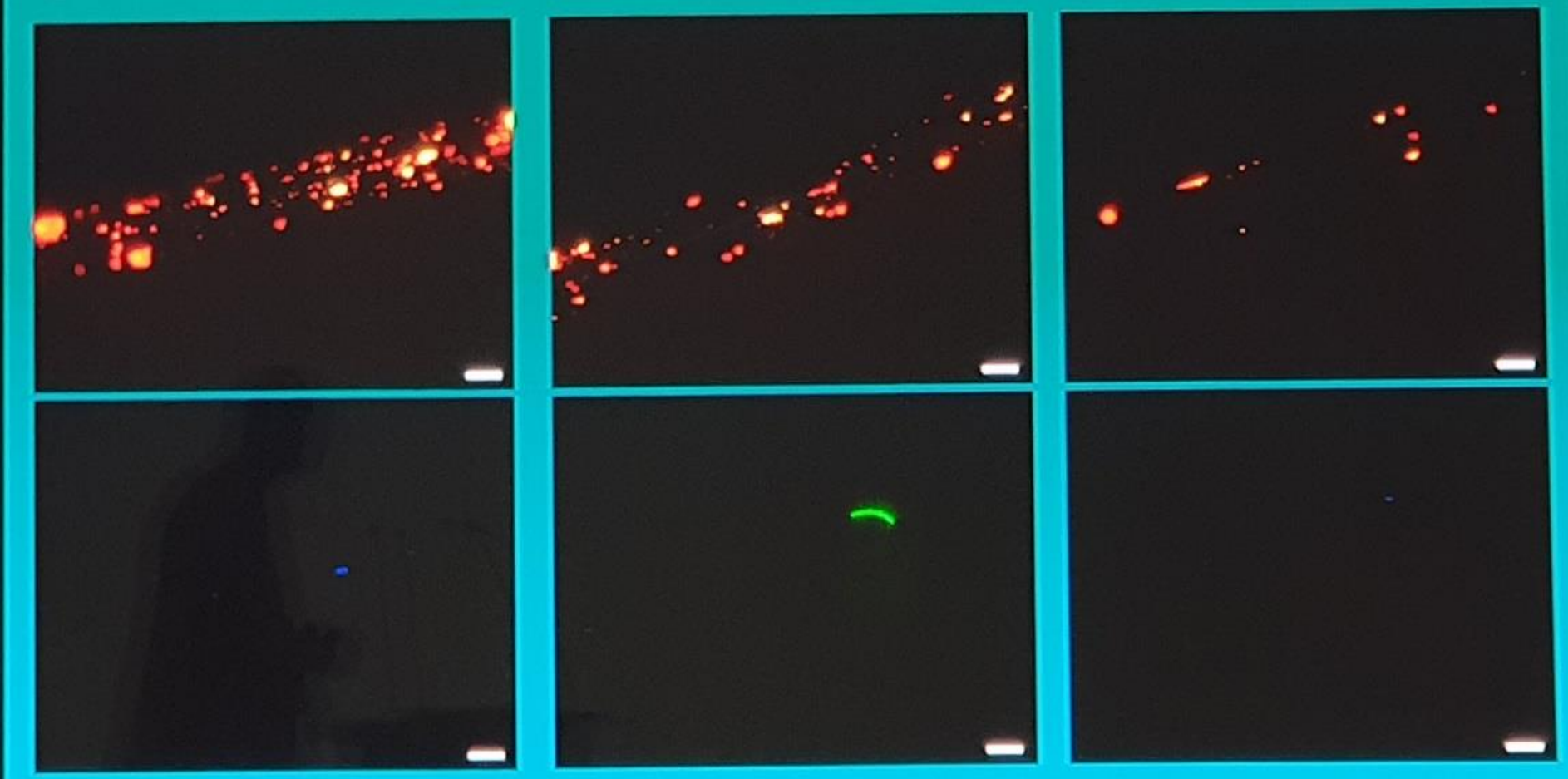
Spiked implant wires after partial CAP treatment



30 sec

60 sec

90 sec

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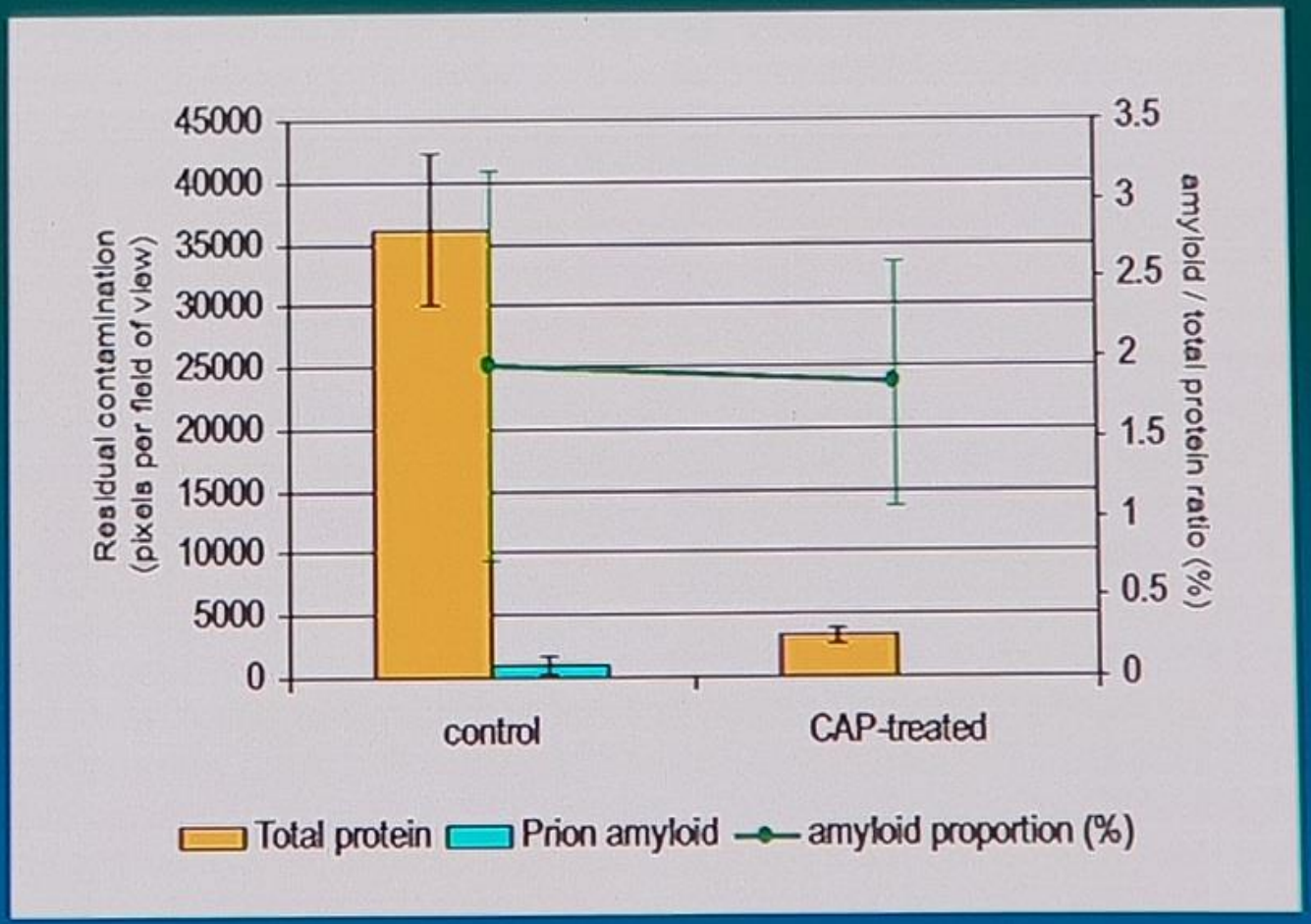
EDIC/EF SR/ThT microscopy:

CAP appears equally effective against amyloid proteins



Pencil-CAP treatment of contaminated wires gives partial reduction in infectivity

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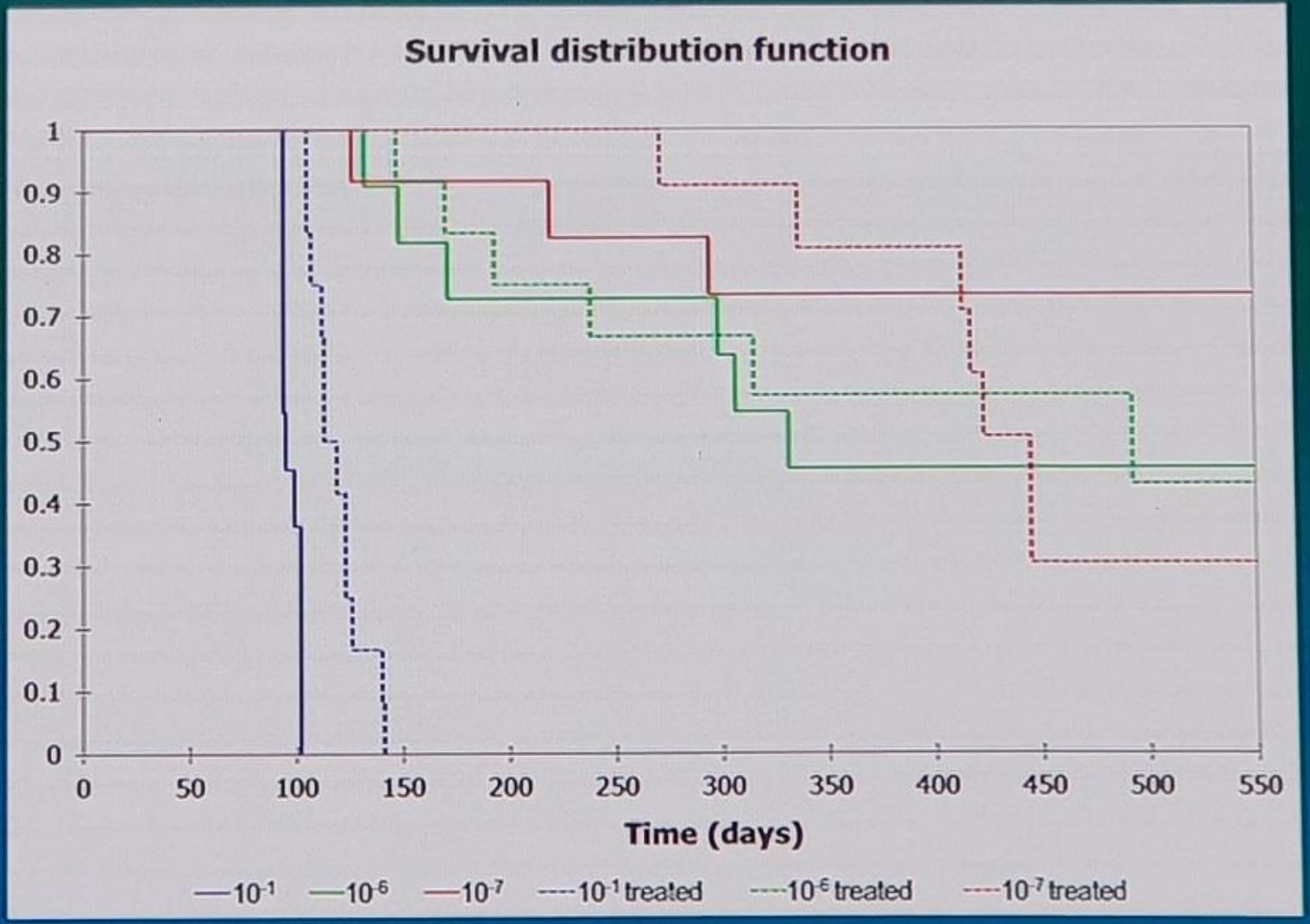

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Cell cuture infectivity assays

Passage study (22L titre)

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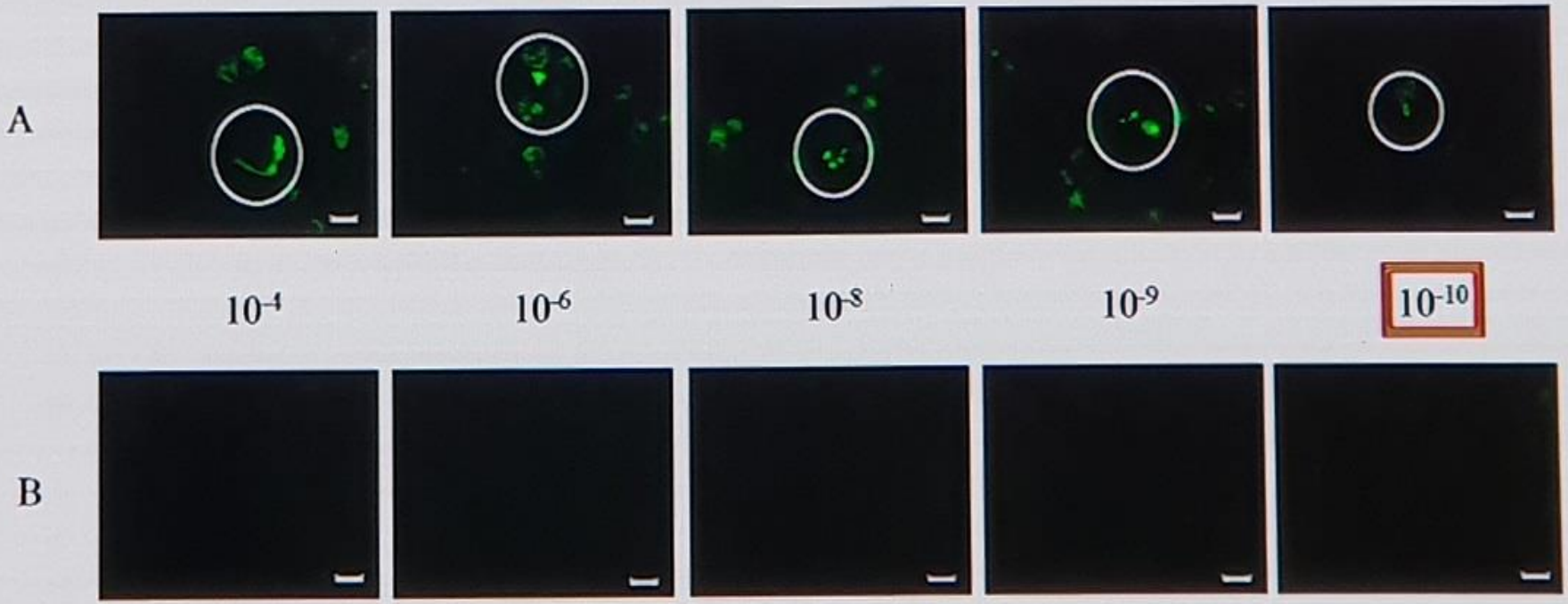
Passage 8 post infection



N2a SB/ThT assay: sensitive dynamic infection range: low as a final 10^{-10} brain dilution

- Enzol very efficient at removing proteins, but not infectivity of remaining protein residues.
- 2 min CAP treatment also inefficient at removing the prion infectivity (gross contamination

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Conclusions

May be better to use CAP after the conventional wash processes, to inactivate any remaining infectious prion residues as a final "polish" procedure.

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