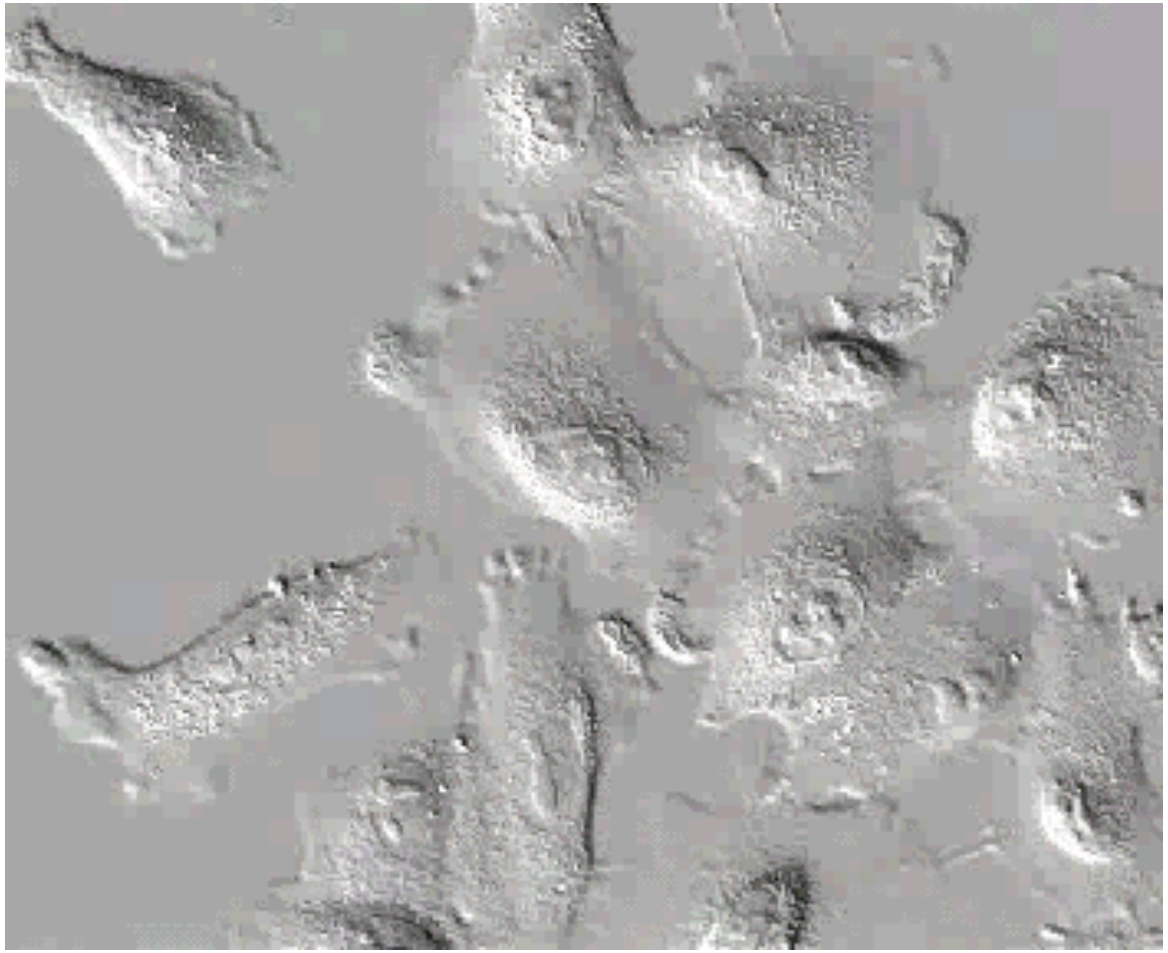
Live cell imaging:
the need for time-resolved microscopy in biology



Peter Lackie - III / BIU

the need for time-resolved microscopy in biology

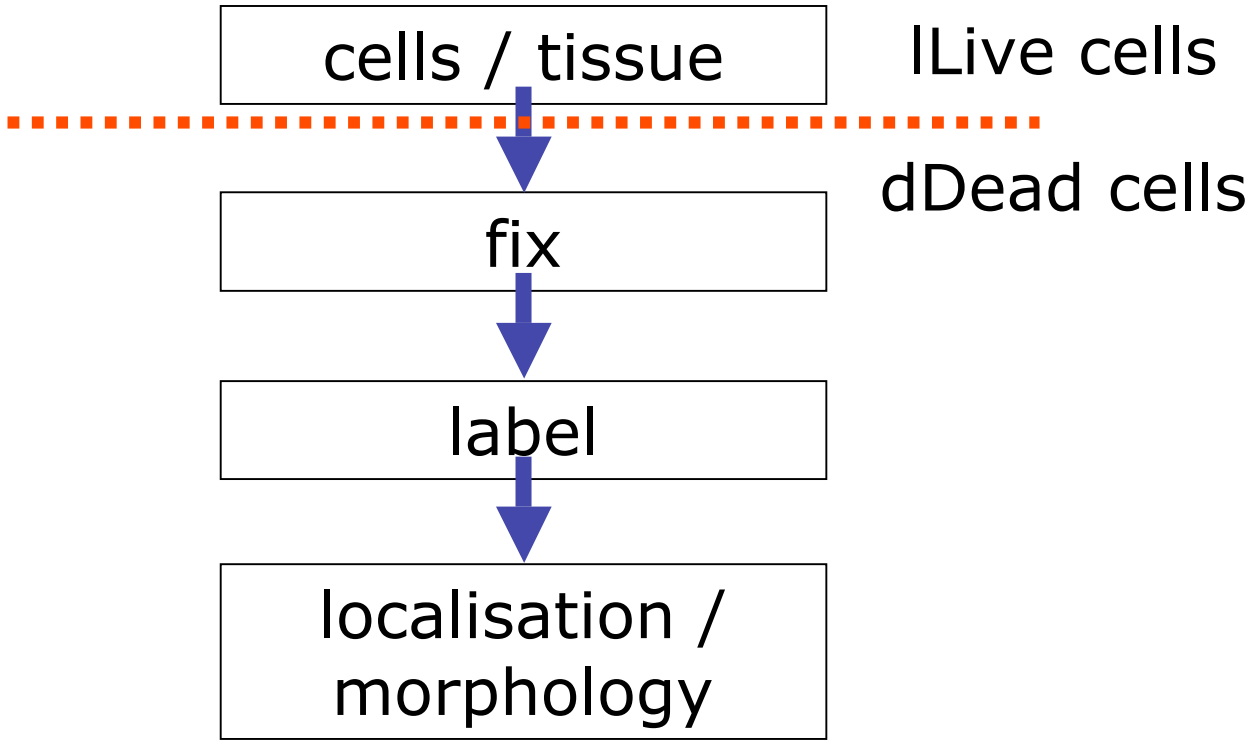
why?

- biological processes have different time-frames
- sequence and dynamics are essential parts of these processes
- detection of rare / unpredictable events

how?

- microscopy and control requirements
- imaging & labelling modes
- data display and analysis

typical tissue processing
(for microscopy)



why?
dynamic processes in biology

- tissue / cell morphology
- localisation and compartmentalisation
- cell-cell interactions
- transient processes
- single cell responses – vs population
- rapid changes / movement e.g. muscle

tissue / cell morphology

- development / repair

localisation and compartmentalisation

- internalisation

cell-cell interactions

- transient processes e.g. stages of differentiation
- single cell responses – especially with minor populations
- infiltrating cells- neuronal contact

rapid changes /movement

- transient processes > 1 Hz - rapid calcium flux
- cilia - fast movement

slow changes / movement

- transient processes - unpredictable & asynchronous events
- slow motility > minutes

live cell imaging- how is it done?

- requirements
- imaging modes for live-cell imaging
- slowing down
- speeding up
- analysis

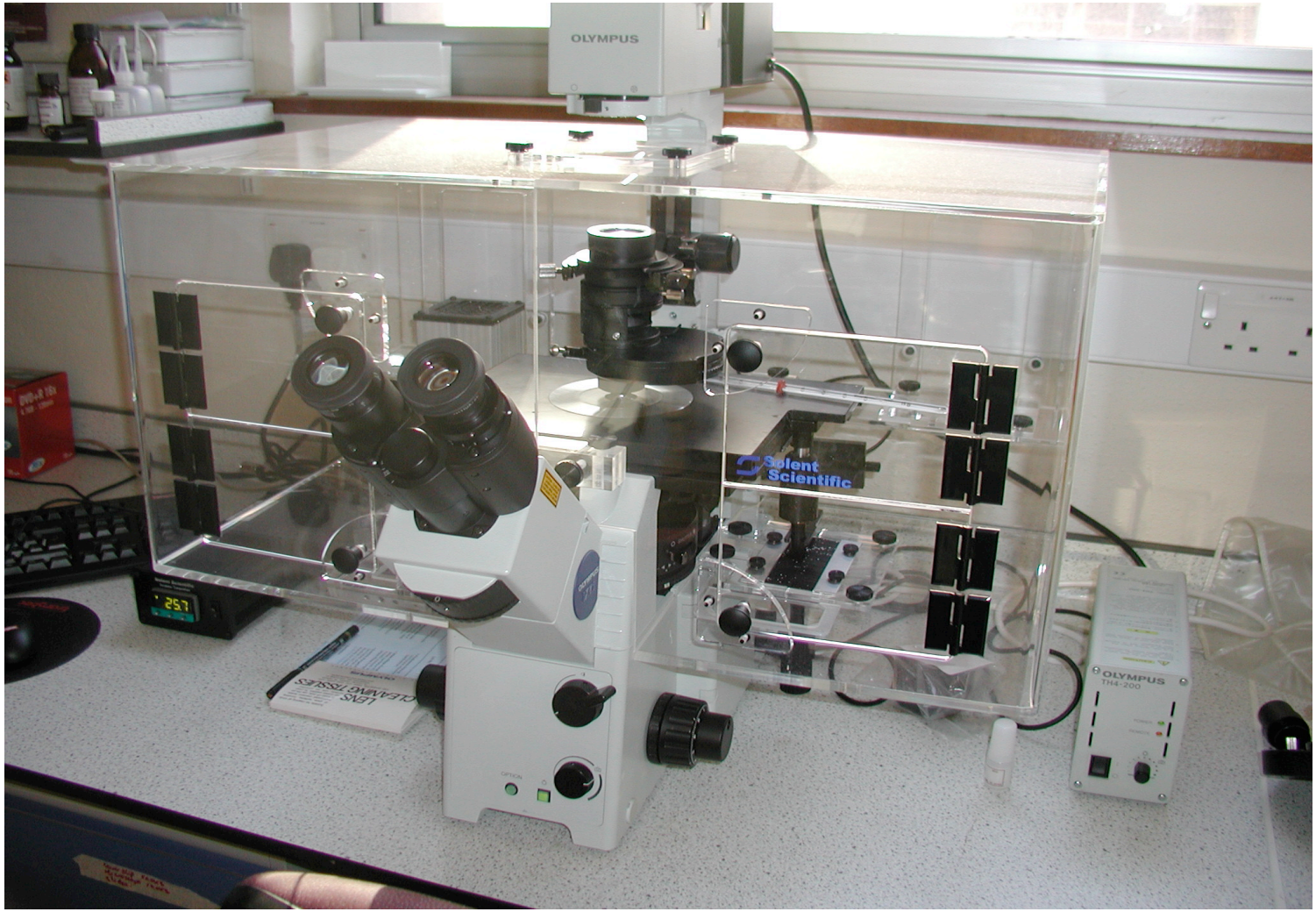
live cell imaging- requirements

essential

- viable cells- environmental chamber – temperature, humidity & CO_2 control
- microscope, camera (digital) + control

useful

- automated microscope for multi-mode imaging (e.g. phase + fluorescence)
- motorised stage for multi-point sequences treatment / control
- minimal light exposure
- autofocus

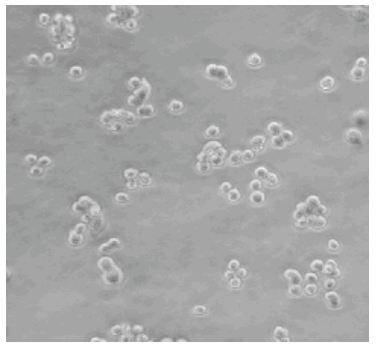


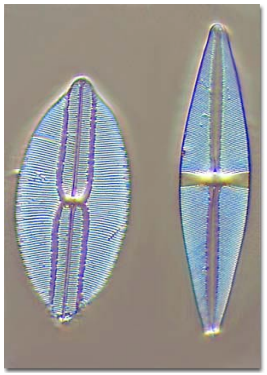
FASTCAM inverted phase contrast microscope

imaging modes for live-cell imaging

unlabelled cells

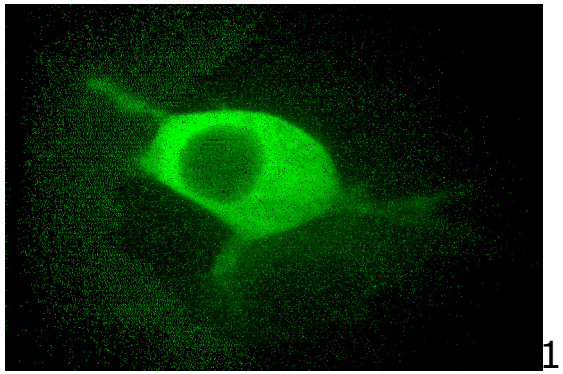
- phase contrast
- differential interference contrast
- relief contrast / Hoffman modulation contrast - especially for cells on plastic





labelled cells / molecules

- bright field / reflected light
- fluorescence, epi-reflection
- confocal



visualisation/ labelling

- unstained cells- altered morphology / autofluorescence
- viable dyes e.g. Hoechst 33258
- labelled probes / beads e.g. phycoerythrin
- tagged molecules e.g. GFP

live cell imaging - some cautions

- demonstrate that cell viability is maintained
- light exposure (especially fluorescence)
- cell behaviour can be altered by labelling e.g. antibodies / lectins

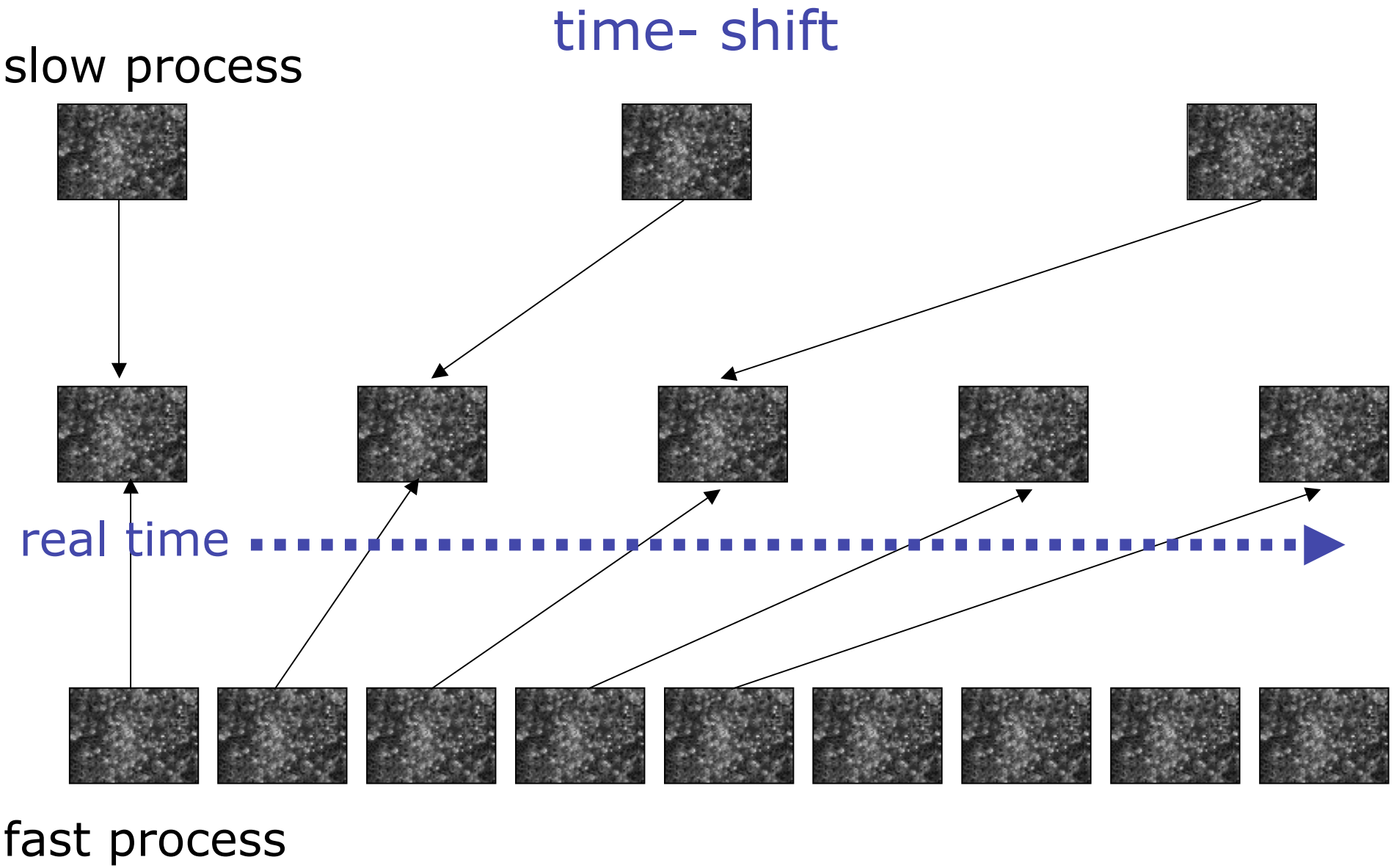

time- shift
slow process
real time
fast process

image analysis & archiving

a typical time-lapse experiment

- phase contrast + single fluorescence
- 3 treatments
- 2 locations in each
- 15 minute intervals
- 24 hours

2 x 3 x 2 x 4 x 24 = **1152 images**
2.5 Mb / image [1280*1024, 16 bit]
1152 x 2.5 = **2.8 Gb … up to 50 Gb**

data storage & analysis implications

analysis

cell / border tracking

- speed, direction, area covered
- comparison with controls

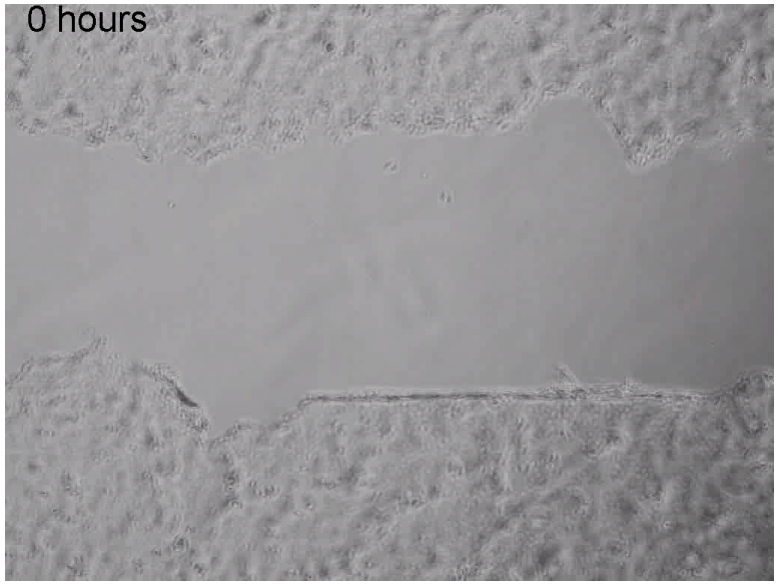
counting

- cell interactions
- cell divisions
- viability
- cell infection

image analysis

- areas
- pattern

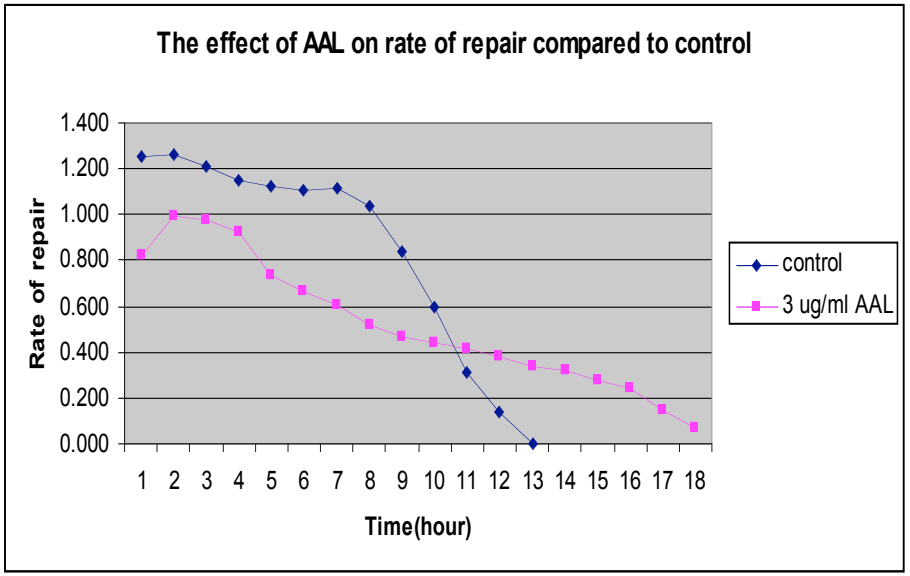
quantifying repair

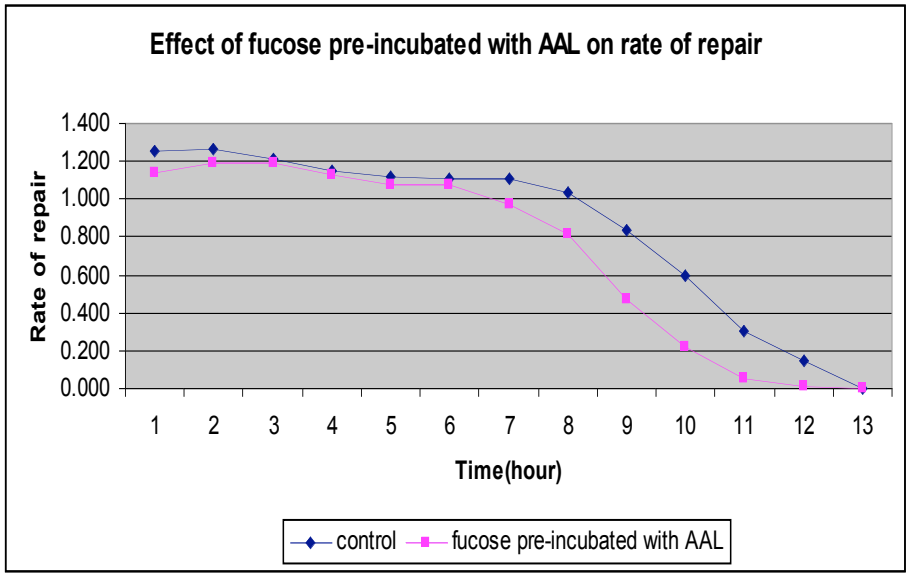


control

treated - AAL

analysis : repair area





change in area of damage per hour,
measured at 60 minute intervals from time-lapse images

conclusion

time-resolved live cell imaging provides information not available by other means

tracing the progress of GFP transfected cells in repair (time lapse sequence)