Confocal Microscopy – Olympus FV1000 Confocal Laser Scanning Microscope.

- Confocal Microscope set up
    - Lasers
        - Argon-ion – multi line 458, 488, 515nm wavelength
        - Helium-neon – Green – 543nm, Red – 633nm
        - UV – 405nm
- Spectral Scan Unit
    - Spectral detection system uses diffraction grating with variable slit – allows the separation of fluorochromes down to 2nm.
- Photomultiplier tube detector (PMT)
    - Amplification of signals
- Pinhole – it's all about the pinhole

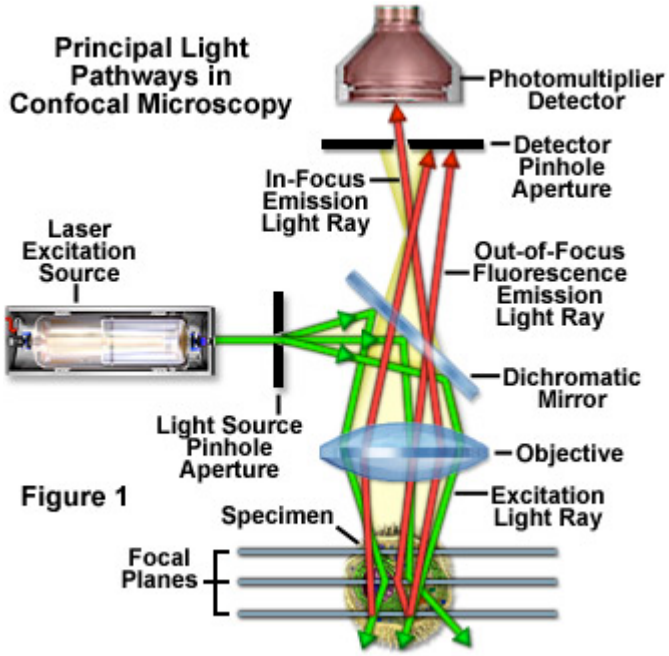

Figure 1

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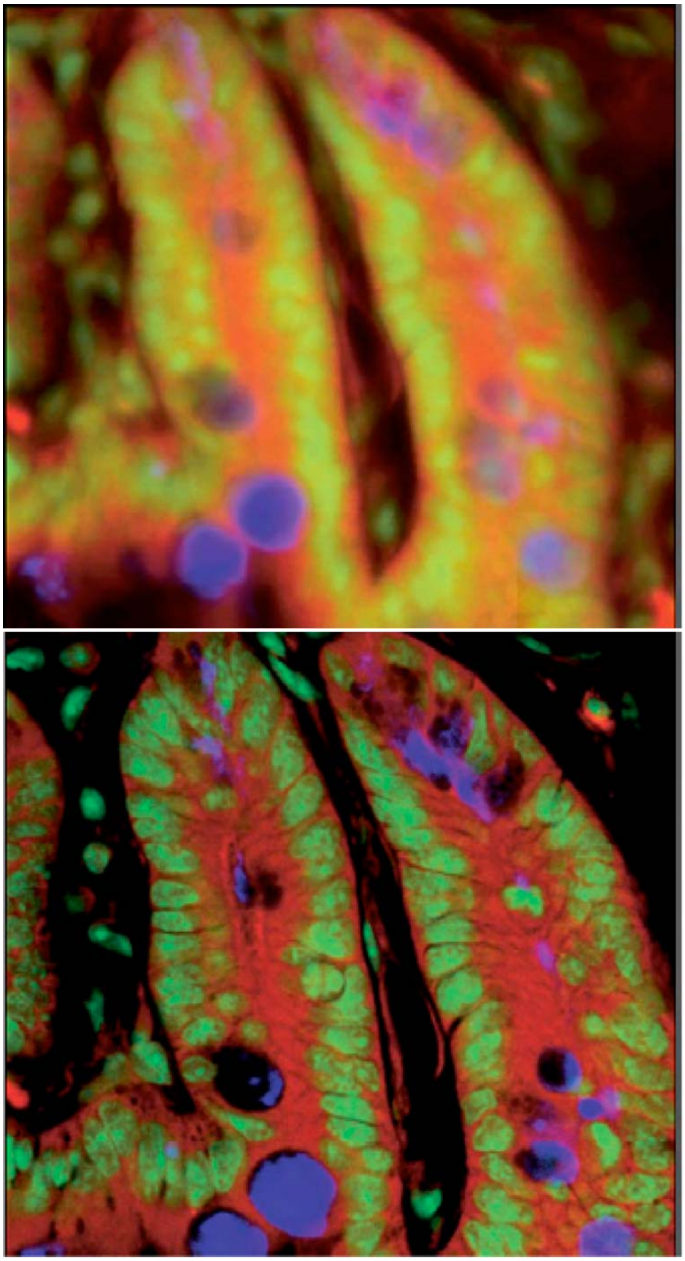
    - (From http://www.microscopyu.com/articles/confocal/confocalintrobasics.html)

- 

(from http://www.dbs.nus.edu.sg/research/facilities/confocal/LSM%20510%20META%20training-DBS-070605.pdf

- Other parts
  - Microscope – upright/inverted
  - PC with a fair bit of memory
  - Optional extras – Live cell setup

- The unique scan head set up allows scanning:
  - XY
  - XYZ (3 dimensions)
  - XZ

- XYt (over time)
- XYZt (over 3 dimensions and time!)
- XYtλ (over time and different wavelengths!!)

- Multiple fluorescent probes
  - Molecular Probes control slide – Bovine pulmonary arterial endothelial cells: DAPI = nucleus, BODIPY = microtubules, TEXAS RED = actin
  - Labelled antibodies
  - GFPs – genetic code known so that genetically fused proteins are possible.

- Applications
  - Standard XY scan – channel separation, co-localization (CONTROL SLIDE)
  - XYZ scan – 3D – produces pretty pictures but little value in analysis (the individual sections provide a lot more information).(ADMIN – SEED)
  - XZ scan – particular useful for the microbiologists to measure colony depth (CHARLTON FILE)
  - XYt – FRAP, FRET and other live cell applications
  - XYtλ – Spectral de-convolution – separation of 2 colours if the wavelengths are known (Admin – RBC Lambda scan image)

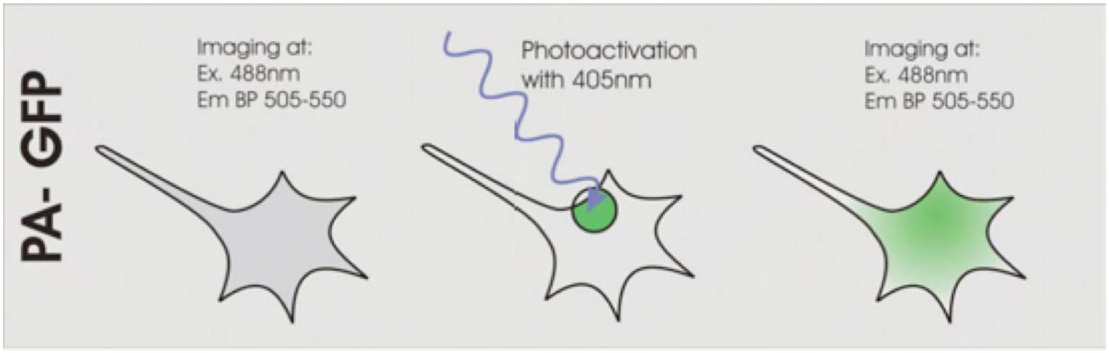
- FRAP – Fluorescence Recovery After Photobleaching
  - Will tell us the amount of protein movement/diffusion over time (ClaireM Image 39)

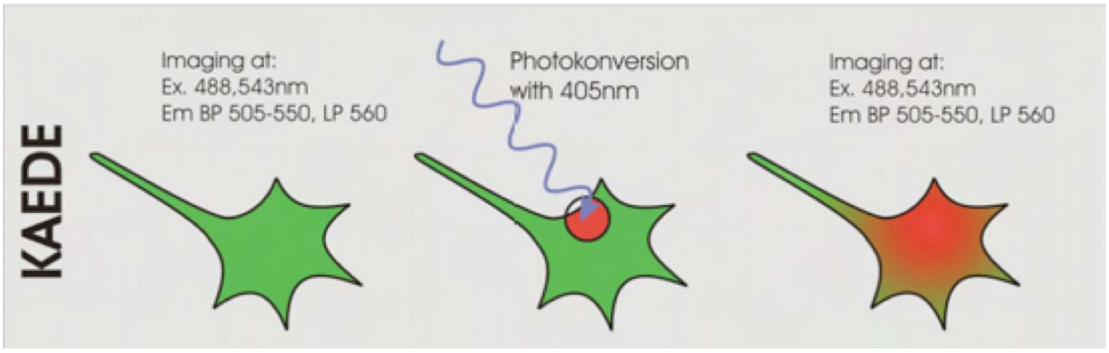
- FRET – Fluorescence Resonance Energy Transfer
  - Will tell us if 2 proteins are likely to be interacting
  - Microscope optical resolution limit = 200nm
  - FRET only occurs within 10nm

- Live Cell Imaging
  - Need humidibox
  - Need 5% CO_2
  - Need anti-vibration table
  - Pros – get to see protein movement and protein-protein interactions as they happen
  - Con – kill the cell, lot of work involved to get excellent images
  - (Kristine File – 260209LCI – YrdC203 RpL12).

- Other interesting confocal stuff:

- Photoactivateble proteins.





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- Photoactivatable GFP – 405nm laser induces the expression of GFP at 488nm – useful for tracking the dynamics of molecular subpopulations within a cell.
- Kaede – Photoconversion from green to red after exposure to 405nm laser - Example: allows delineation of a single neuron in a dense culture

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References:

http://www.dbs.nus.edu.sg/research/facilities/confocal/LSM%20510%20META%20training-DBS-070605.pdf

http://www.olympusmicro.com/primer/techniques/confocal/index.html

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