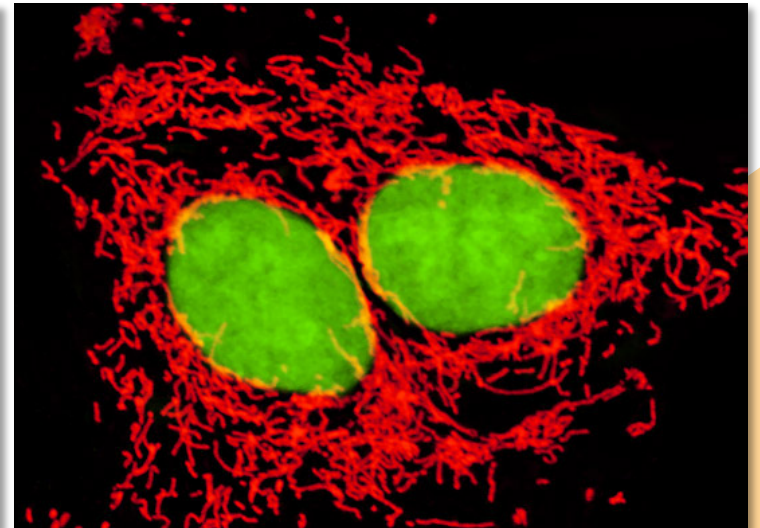
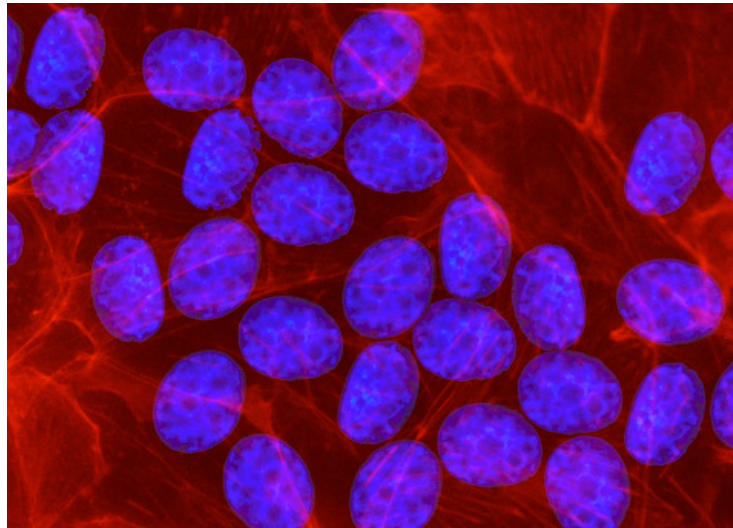
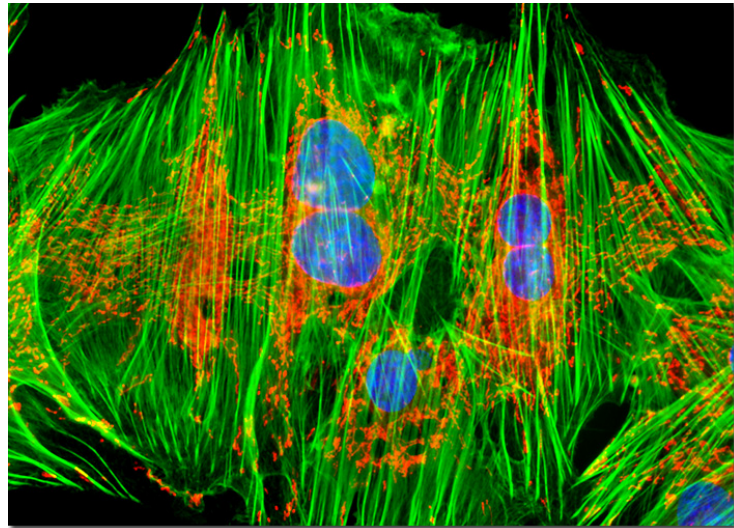
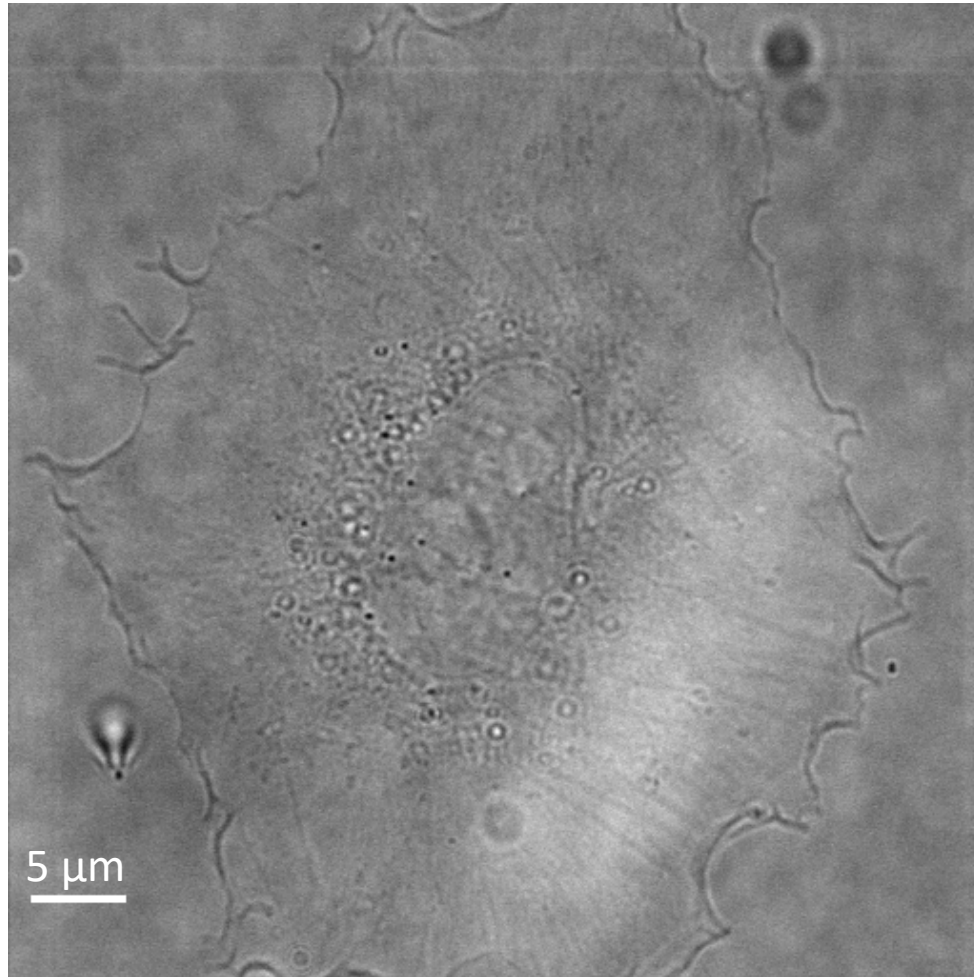


Microscopia de fluorescência e confocal



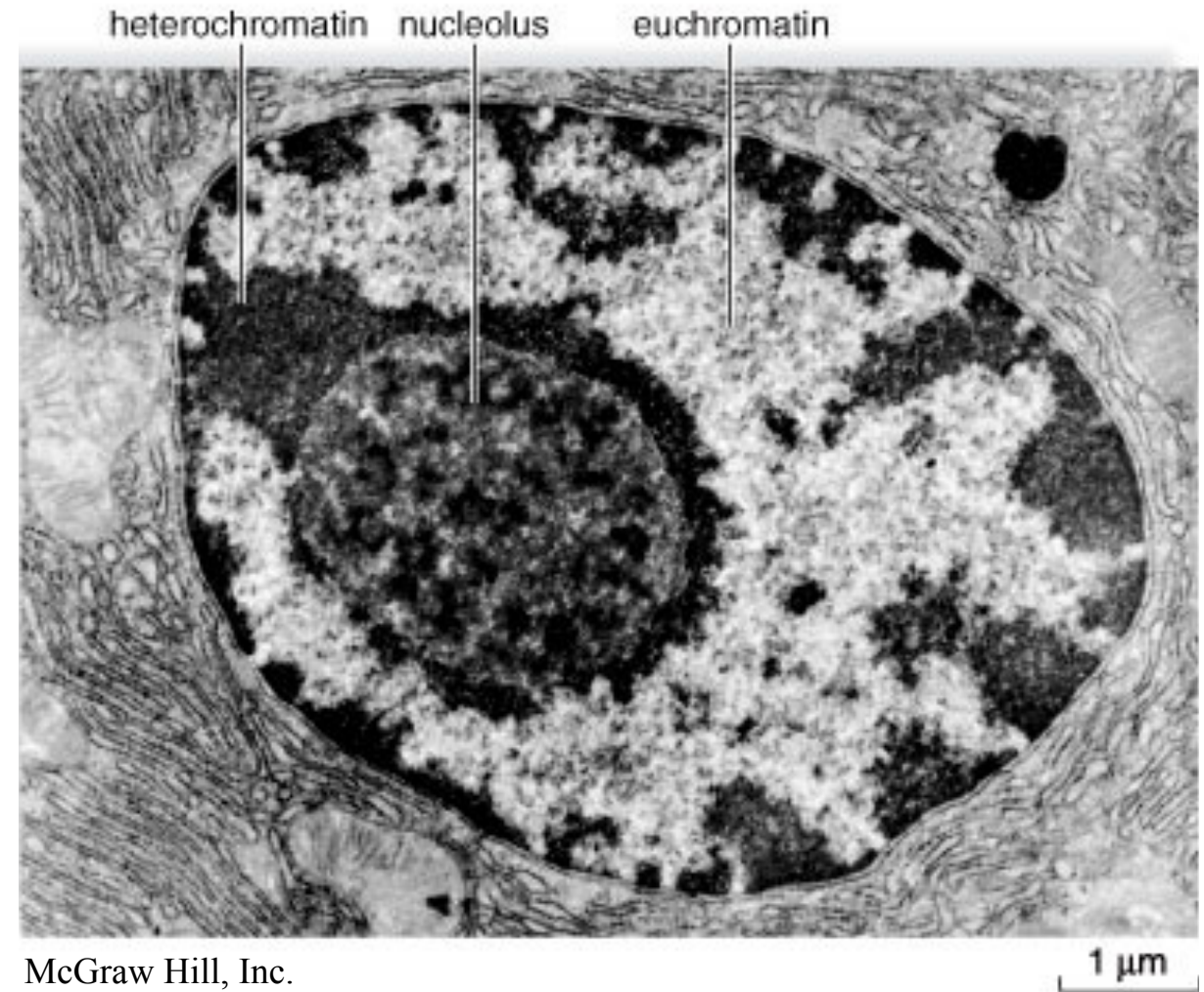
Bright Field (BF) and Electron Microscopy (EM)

A Köhler, Zeitschrift für wissenschaftliche Mikroskopie, 1893



- **Non invasive**
 - **Live cells**
 - **Lack of contrast**
 - **Lack of specificity**
 - **Low resolution**
- (~1900)

M Knoll, E Ruska, Zeitschrift für Physik, 1932



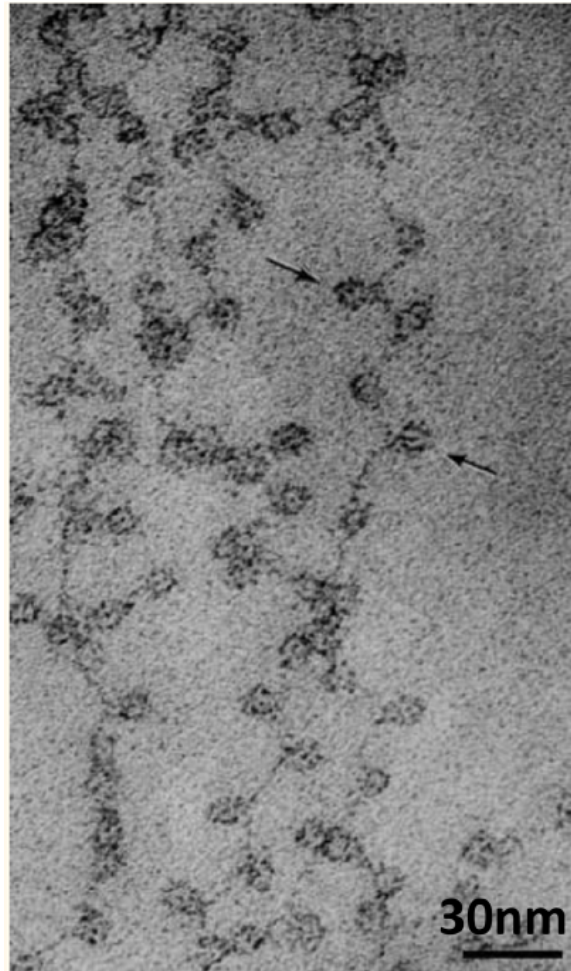
McGraw Hill, Inc.

- **Resolution (~1 nm)**
 - **Contrast**
 - **Lack of specificity**
 - **Fixed samples**
- (~1940)

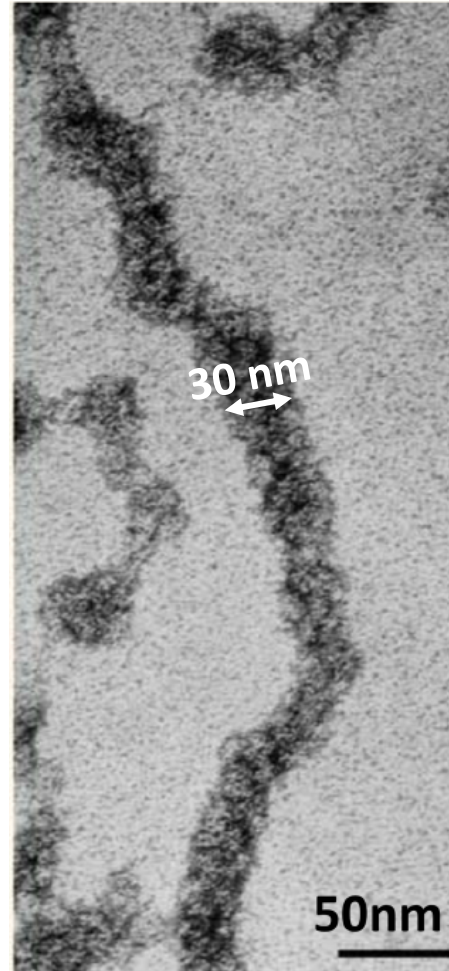
Scanning Electron Microscopy (SEM)

D Attack and KCA
Smith, Pulp Paper
Canada, 1956

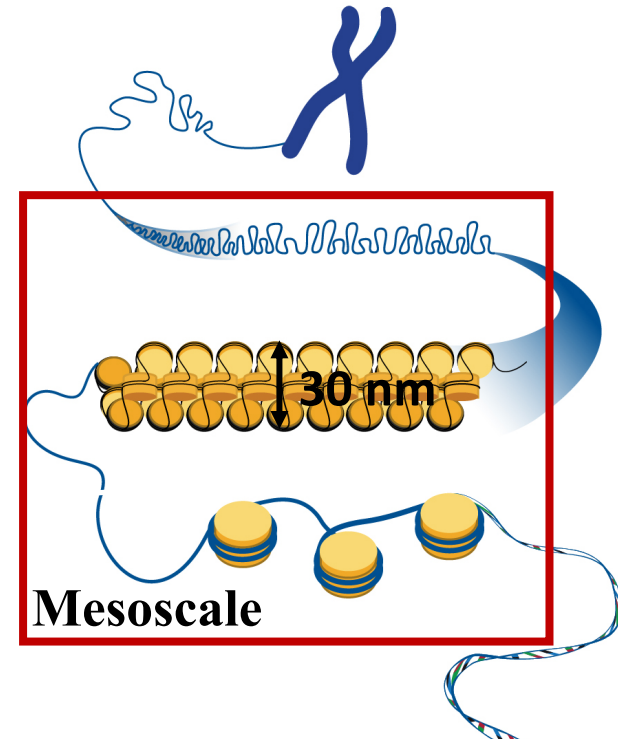
SEM images of chromatin in vitro



Bead on a string



30-nm fiber



(~1980)

P Oudet et al., Cell,
1975

Finch et al., PNAS,
1976

Woodcock et al., J. Cell
Biol., 1984

Olins et al., Nature Reviews Molecular Cell Biology, 2003

- Resolution (~1 nm)
- Contrast
- Lack of specificity
- Fixed samples

Fluorescence Microscopy

Microtubules

JFW Herschel, Philos Trans R Soc
London, 1845

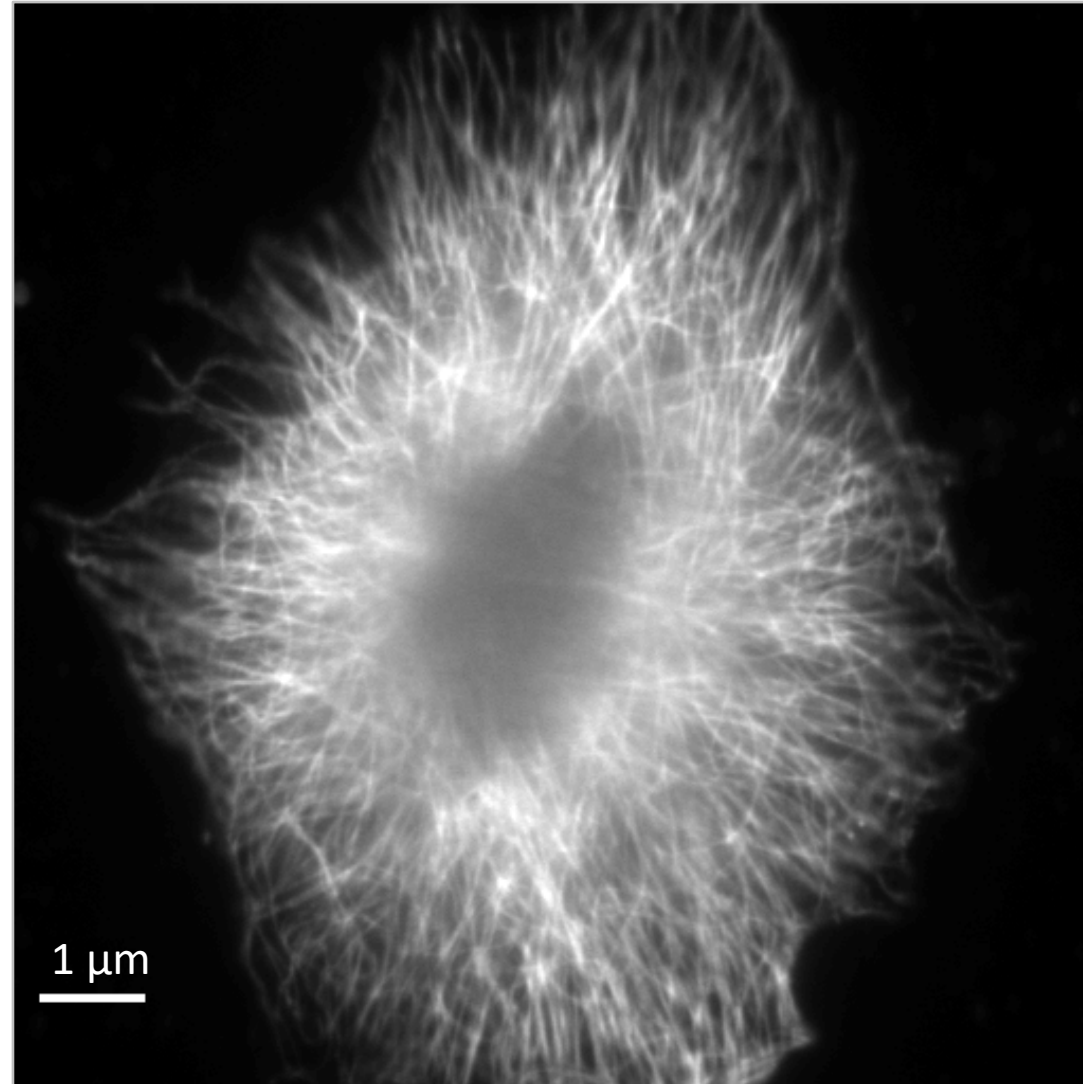
GG Stokes, Philos Trans R Soc
London, 1852

OD Heimstadt, Z wiss Mikr, 1911

AH Coons et al., J Immunol, 1942

DC Prasher et al., Gen, 1992.

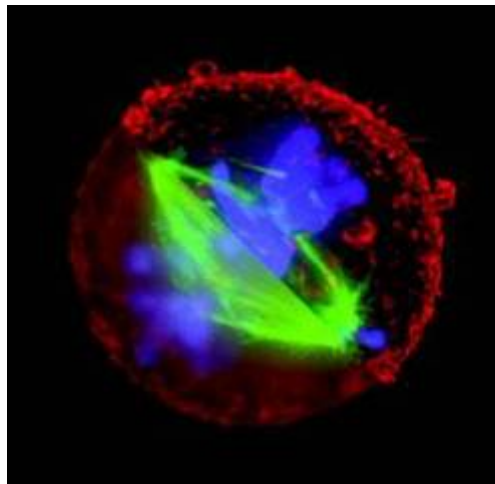
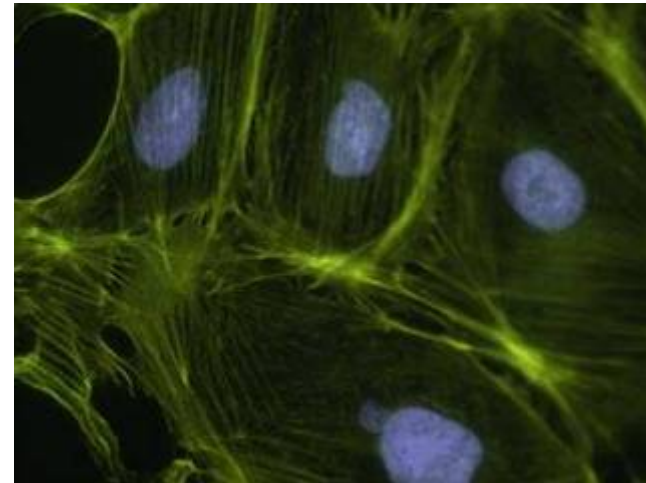
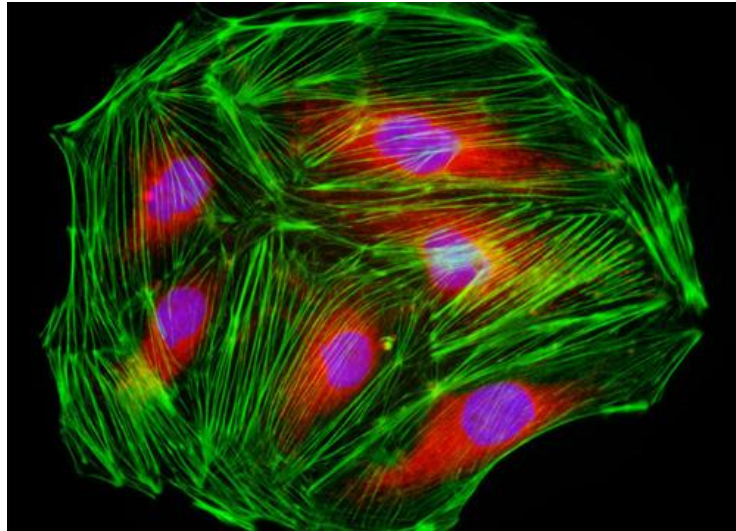
(~1950)



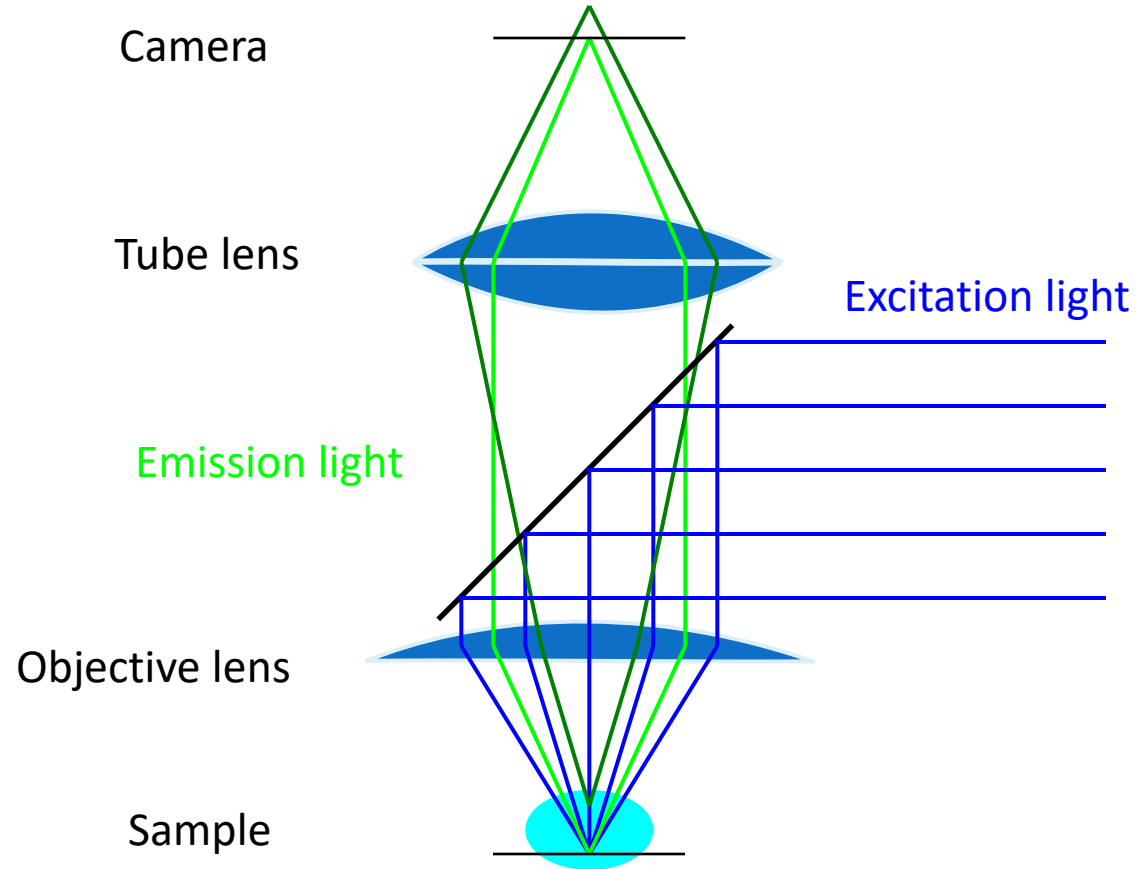
- **Specificity**
- **Contrast**

- **Low spatial resolution (~250 nm)**

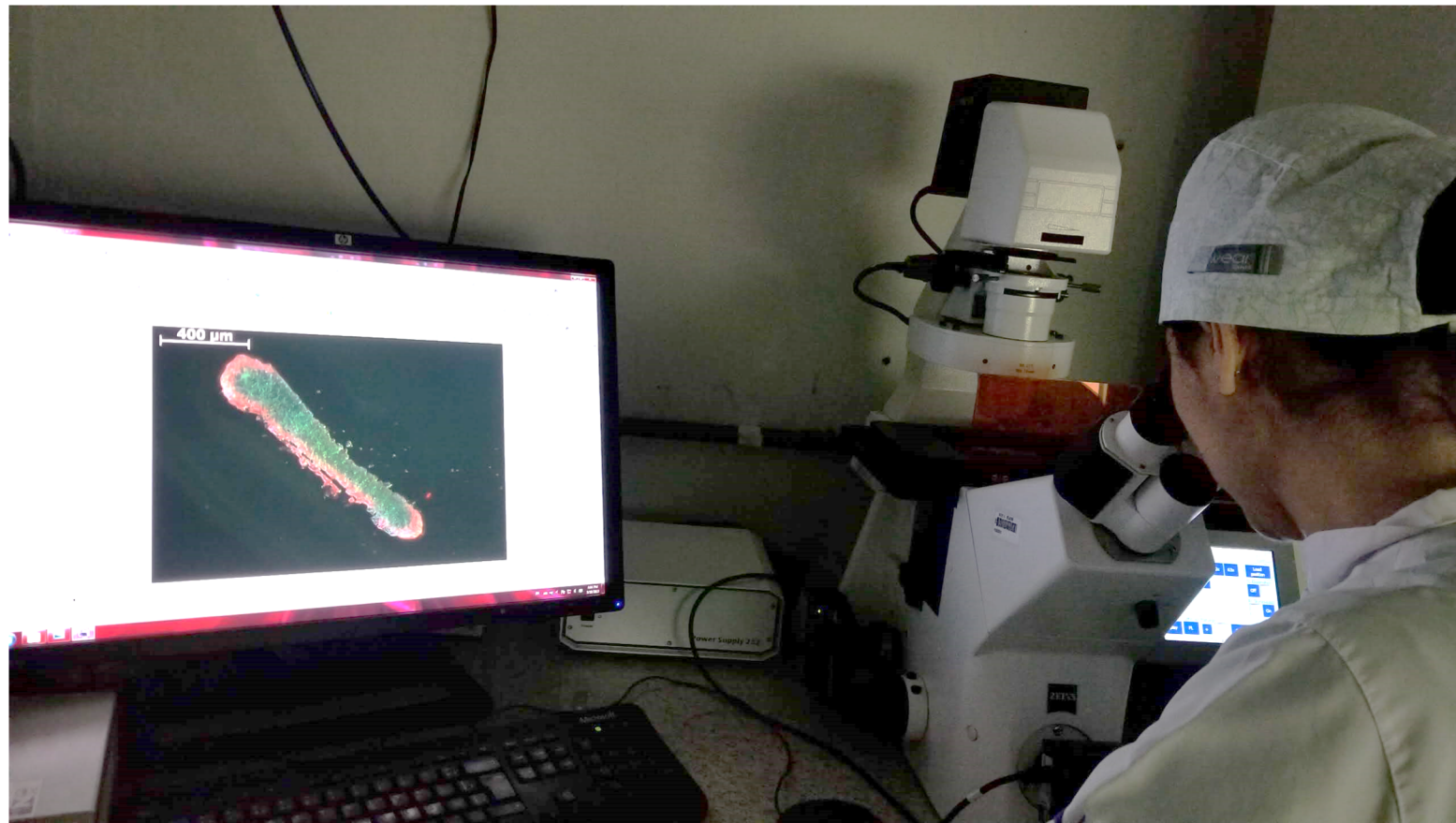
Microscopia de fluorescência



Microscópio de fluorescência

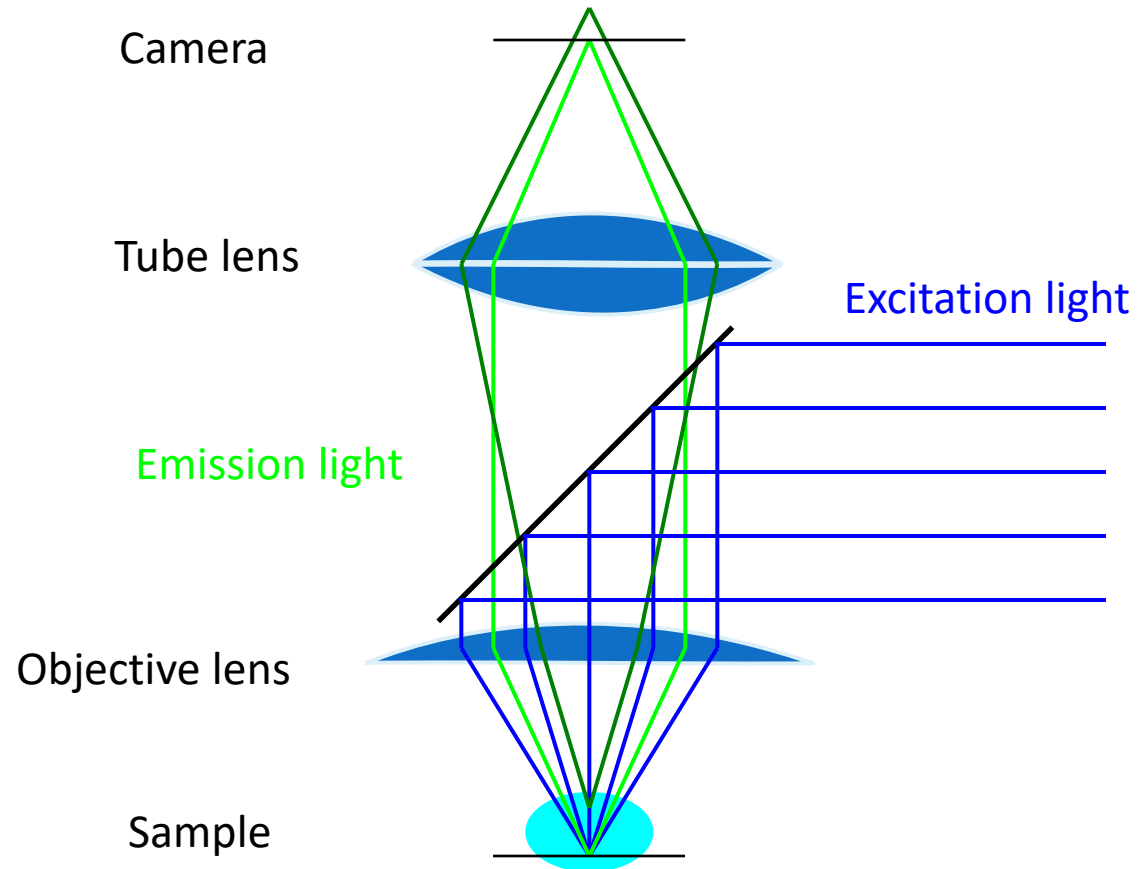


Introdução a Microscopia de fluorescência



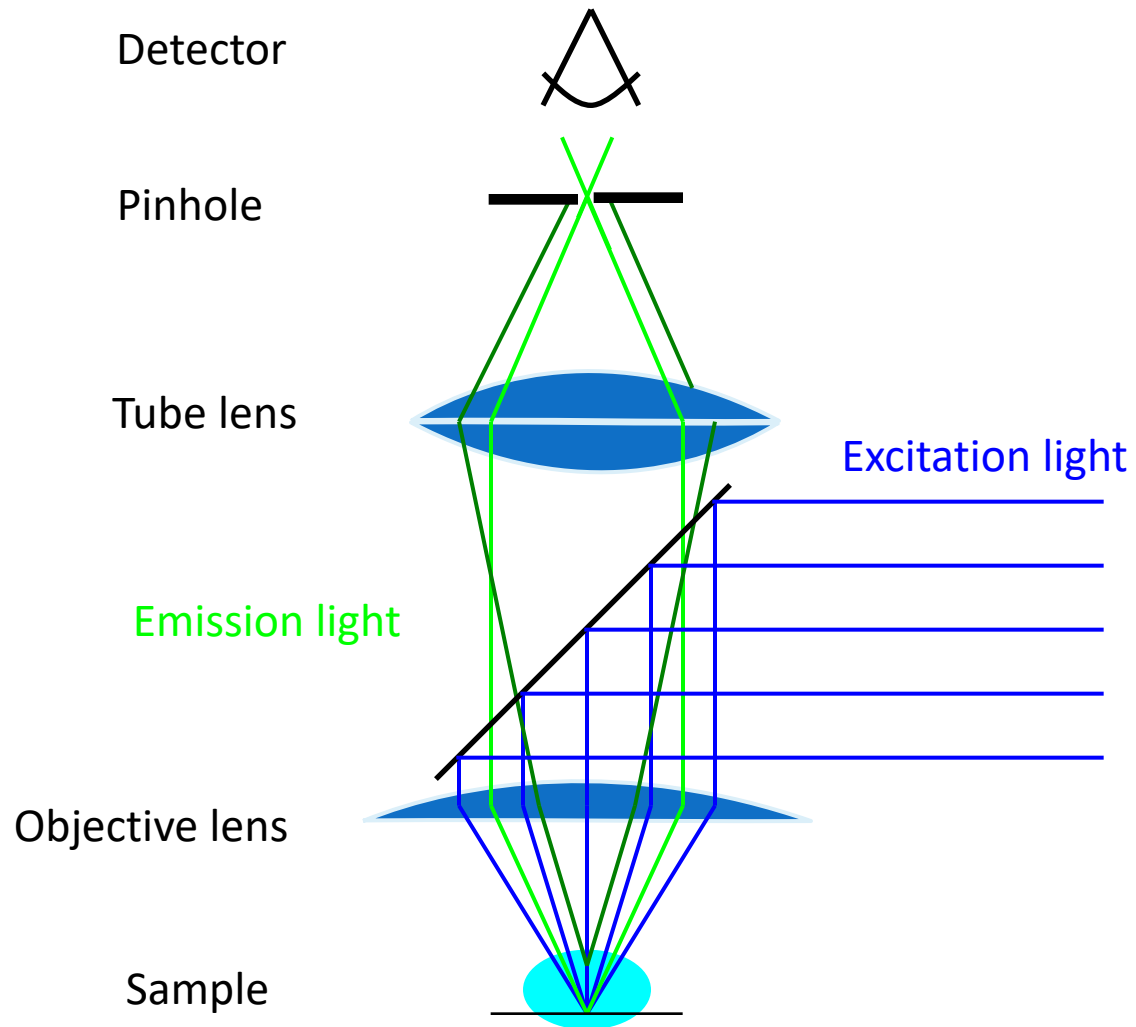
<https://www.youtube.com/watch?v=PCJ13LjncMc>

Microscópio de fluorescência



Problema: fluorescência é emitida em todo o caminho óptico na amostra

Microscópio confocal



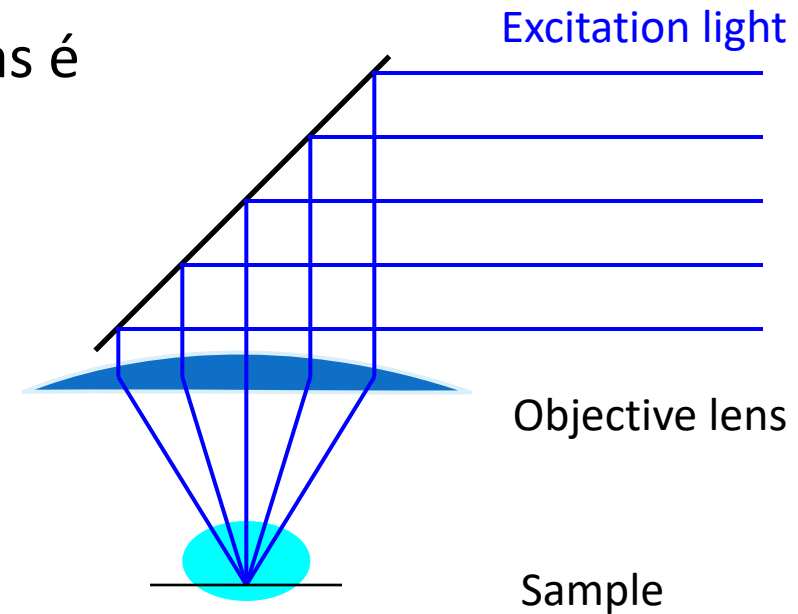
Varredura da excitação ponto-a-ponto para formar a imagem.

Fonte de luz

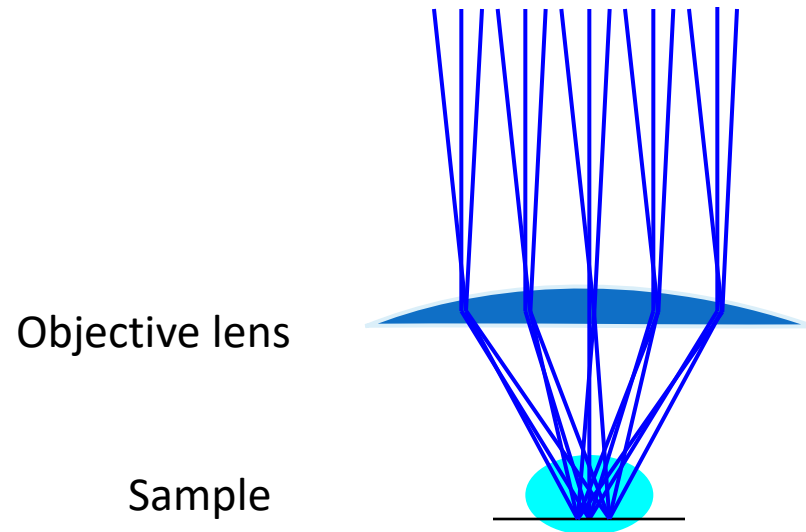
Luz de excitação deve ser focalizada em um “diffraction limited spot”

Poderia ser empregado lâmpada, mas é pouco eficiente

Laser: perfeitamente colimado e alta potência



Varredura



Modificação do ângulo de entrada do laser altera sua posição no plano da amostra

O ponto de emissão muda, então se deve assegurar a coincidência espacial do pinhole.

Varredura laser

