

EC Imagerie et Traceurs

**De la microscopie confocale in vitro
A la microscopie confocale in vivo**

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FLUORESCENCE

- La **fluorescence** est une émission lumineuse provoquée par l'excitation d'une molécule (généralement par absorption d'un photon) immédiatement suivie d'une émission spontanée. Ce processus est différent de la phosphorescence. La fluorescence peut entre autres servir à caractériser un matériau.

FLUORESCENCE

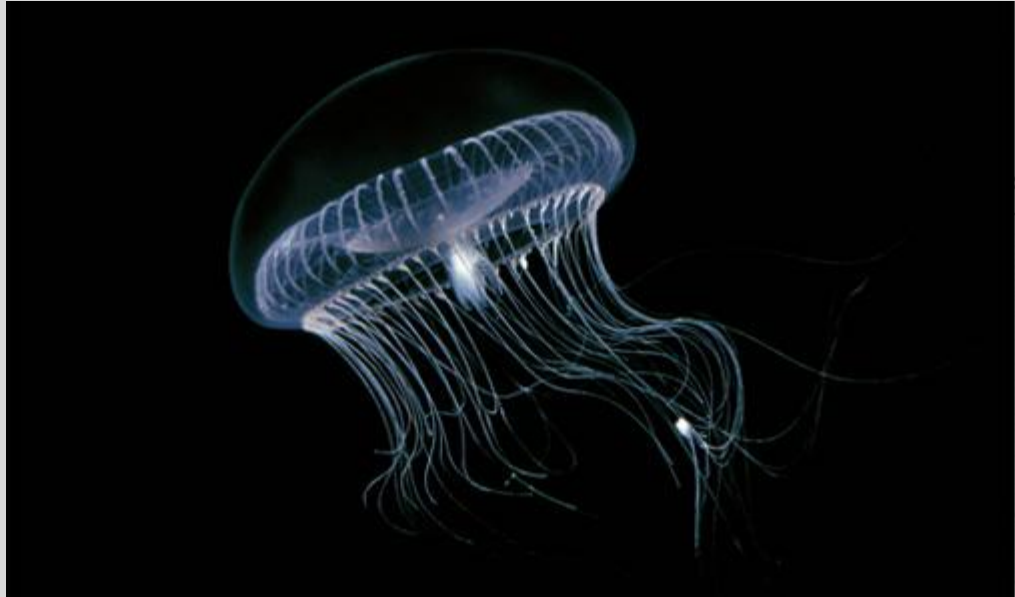


Photo of Aequorea Victoria, Kevin Raskoff

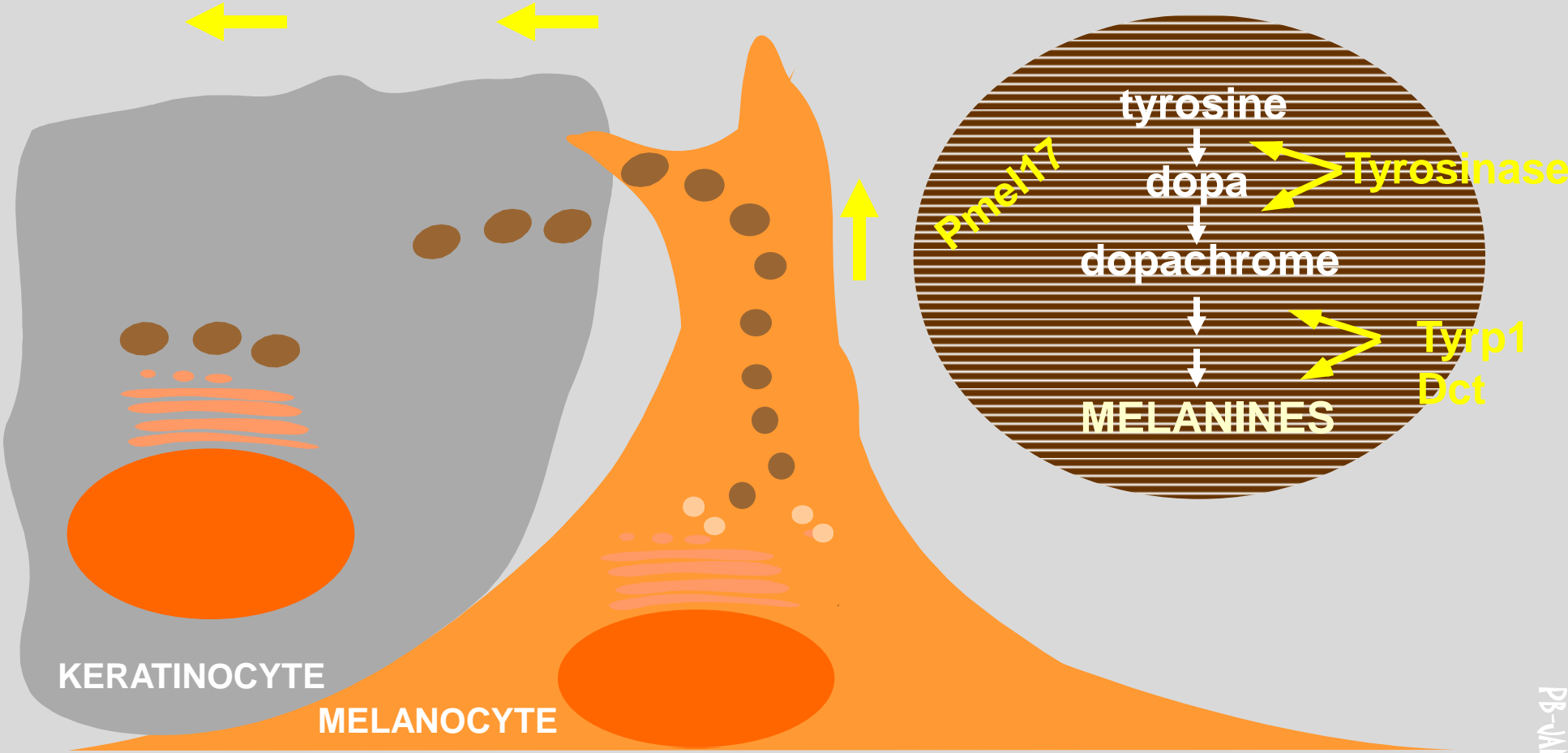
GFP (green fluorescent protein)

Syndrome de Griscelli-Prunieras

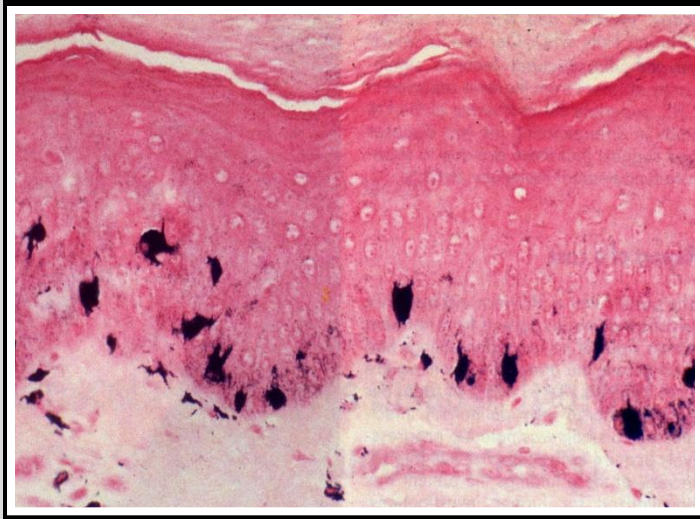


- Albinisme
- Déficit immunitaire
- Troubles neurologiques (retard psycho-moteur)

Pigmentation Mélanique Cutanée



Syndrrome de Griscelli-Prunieras



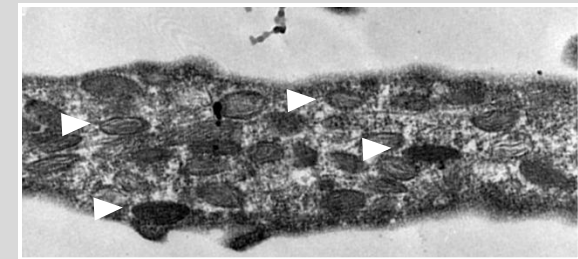
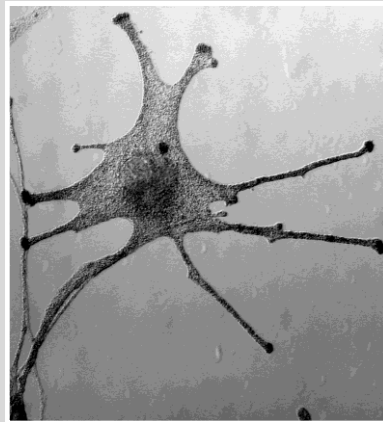
Distribution anormale des mélanosomes dans les mélanocytes «SGP»

Microscopie optique

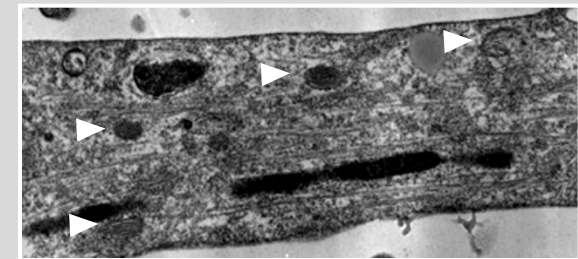
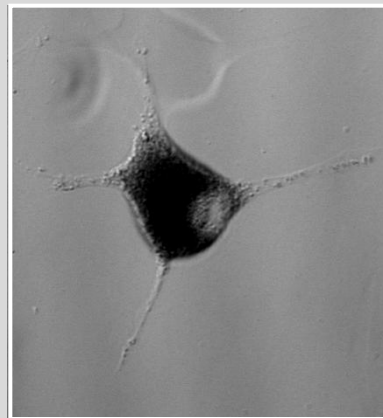
Immuno-Fluorescence

Microscopie électronique

Melanocyte normal



Melanocyte « SGP »



Syndrome de Griscelli-Prunieras (type 1)

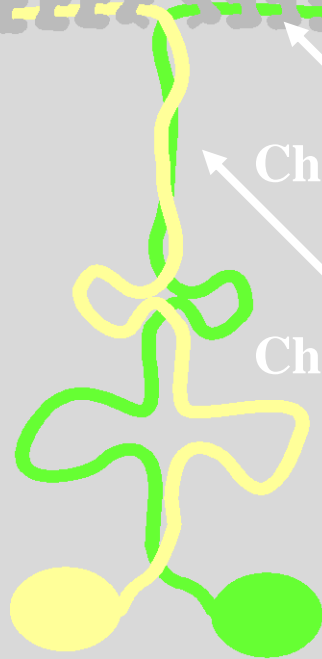
Implication de la Myosine Va

Domaine N-terminal
(liaison actine)



Chaines légères

Chaines lourdes



Domaine C-terminal
(liaison organites)

Le gène du SGP est en 15q21, et certains patients « SGP » ont des mutations du gène MYOVA.

Pastural E. et al. Griscelli disease maps to chromosome 15q21 and is associated with mutations in the myosin-V gene. *Nat Genet.* 1997. 16: 289-292.

La myosine-Va est un moteur moléculaire des mélanosomes.

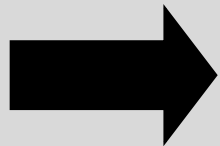
Wu X. et al. Myosin-V associates with melanosomes in mouse melanocytes: evidence that myosin-V is an organelle motor. *J Cell Sci.* 1997. 110: 847-859.

Transport des mélanosomes

Un deuxième gène?

Certains patients « SGP » n'ont pas de mutations de *MYOVA* (15q21), mais d'un autre gène (également situé en 15q21!).

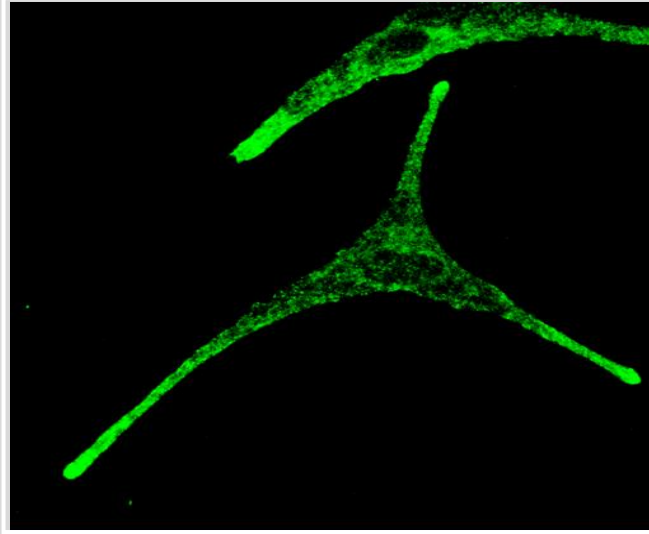
Le gène humain de la petite GTPase Rab27a est situé en 15q21.



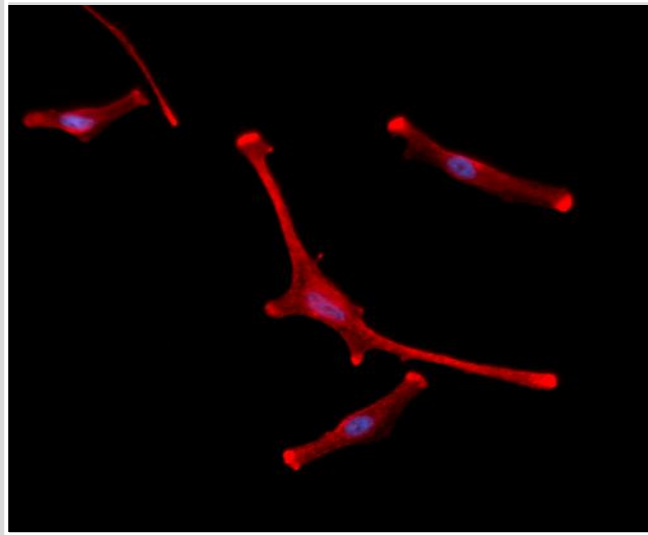
Etude de Rab27a dans les mélanocytes

Rab27a co-localise avec les mélanosomes

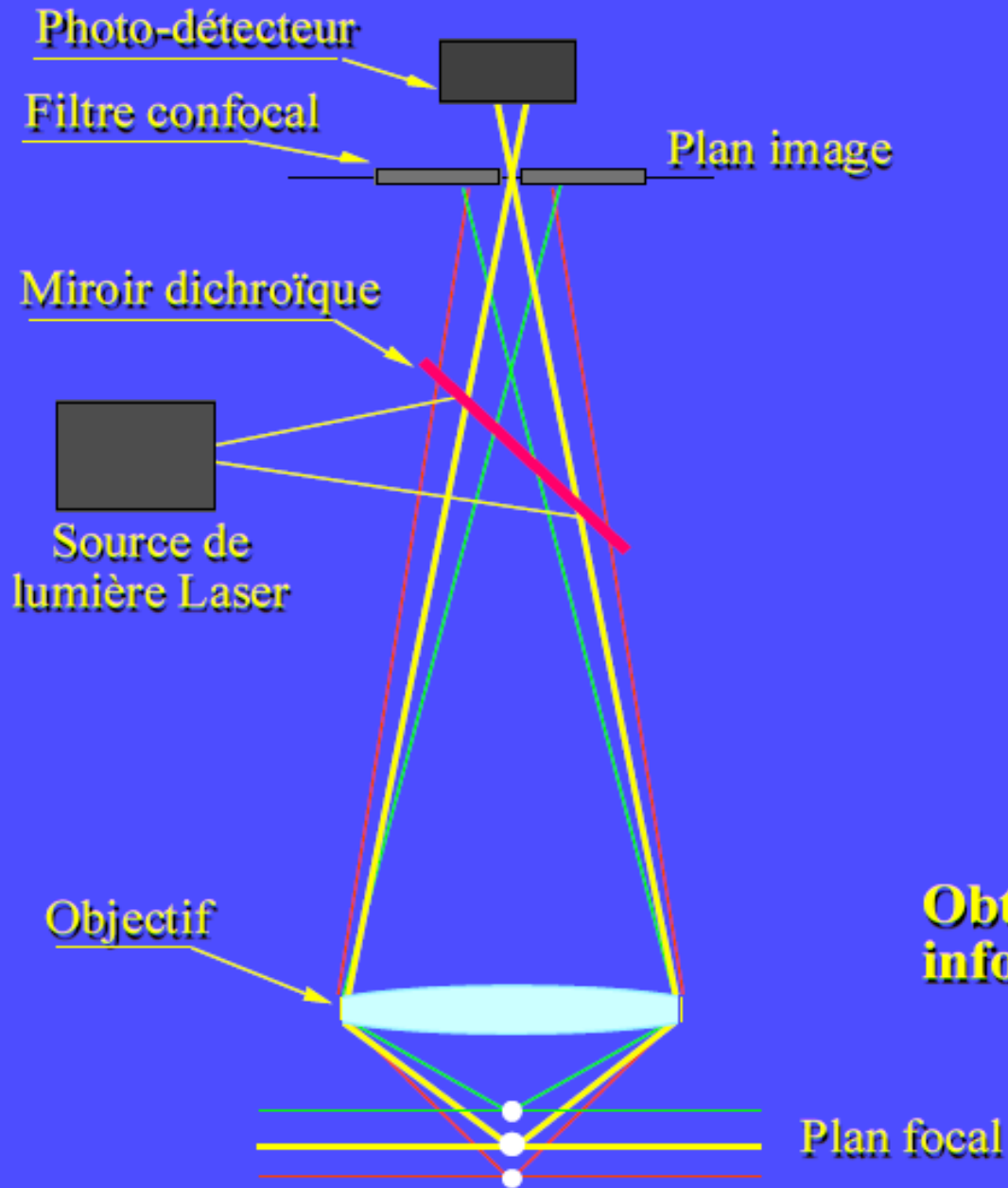
Myosine-V



Rab27a



Microscope confocal à balayage laser



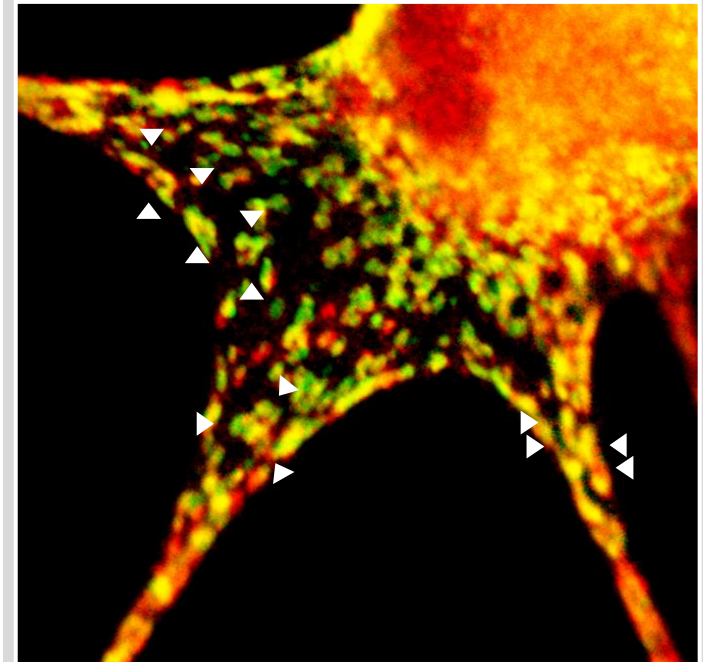
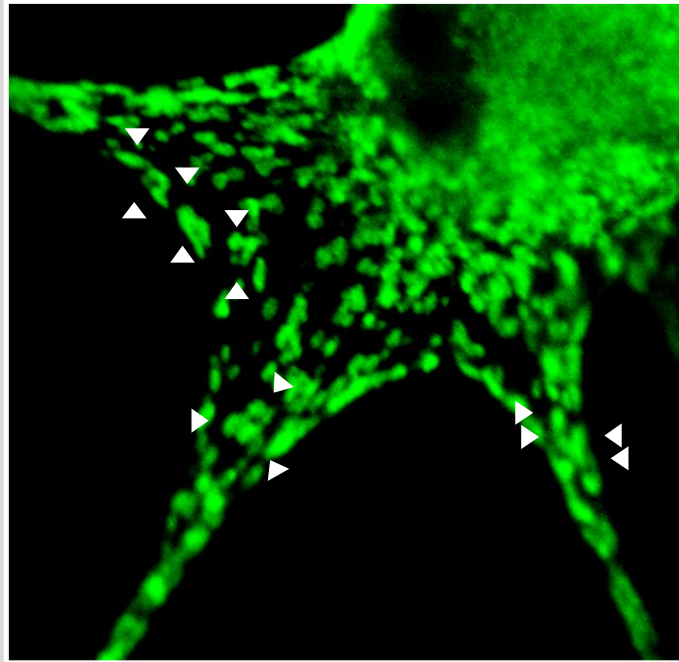
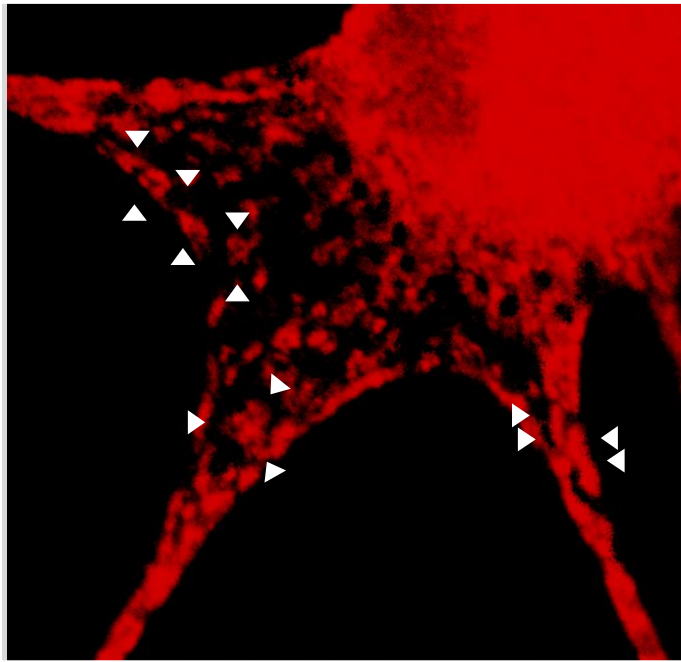
Obtention directe de coupes optiques sans information parasite des plans voisins

Rab27a co-localise avec les mélanosomes

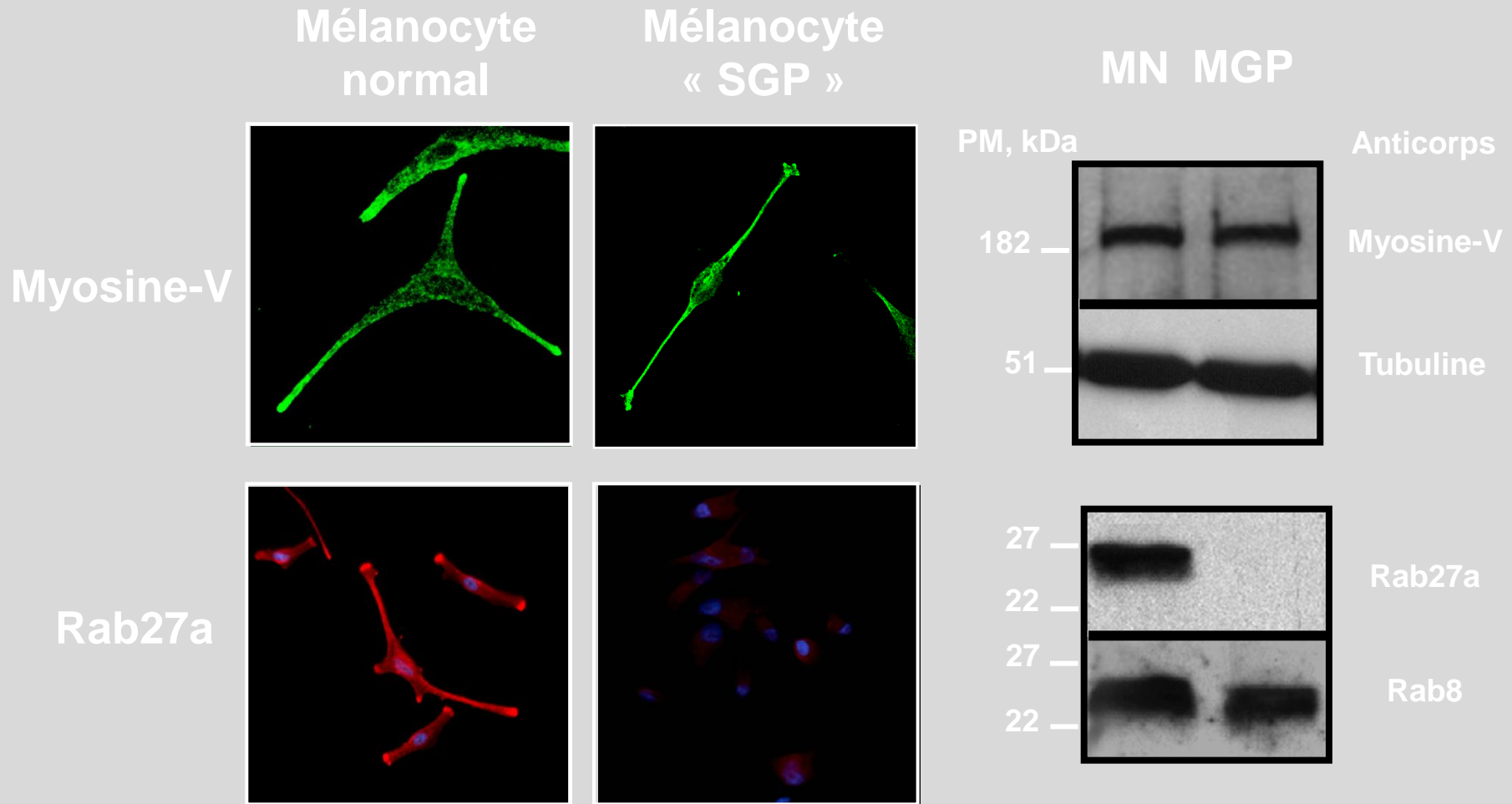
Tyrp1

Rab27a

Tyrp1+Rab27a



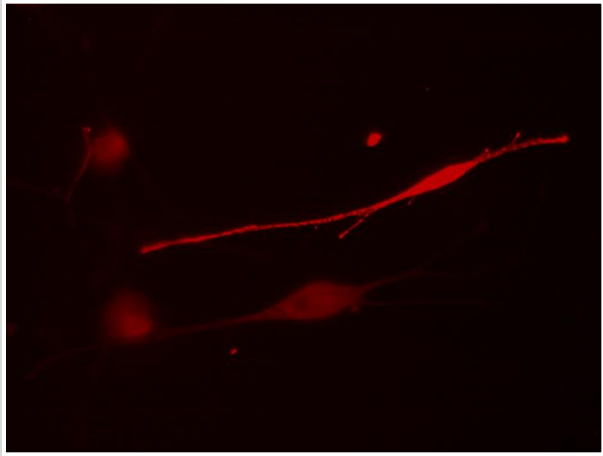
Absence de Rab27a dans les mélanocytes «SGP»



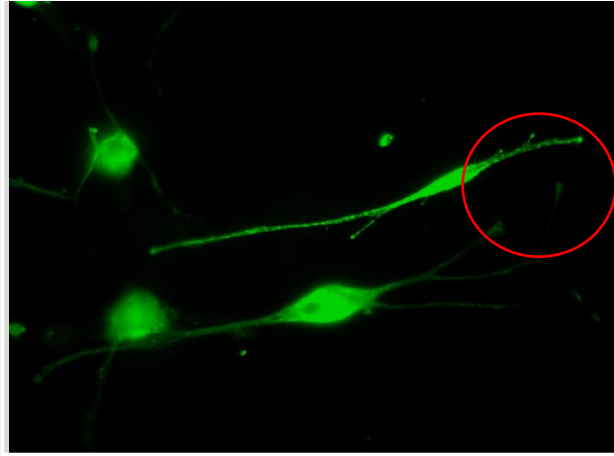
Mutations de
Rab27a

Expression de Rab27a dans les mélanocytes «SGP»

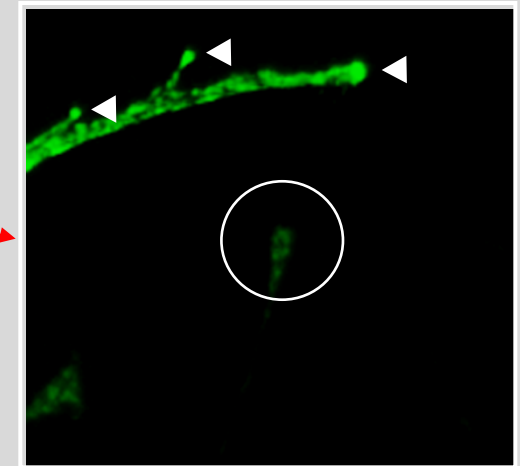
Rab27a



Mélanosomes

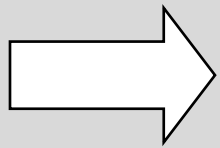


Mélanosomes

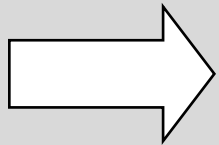


Conclusions

L'albinisme de certains patients atteints du syndrome de Griscelli-Prunieras est dû à un défaut de transport des mélanosomes à cause de l'absence de Rab27a

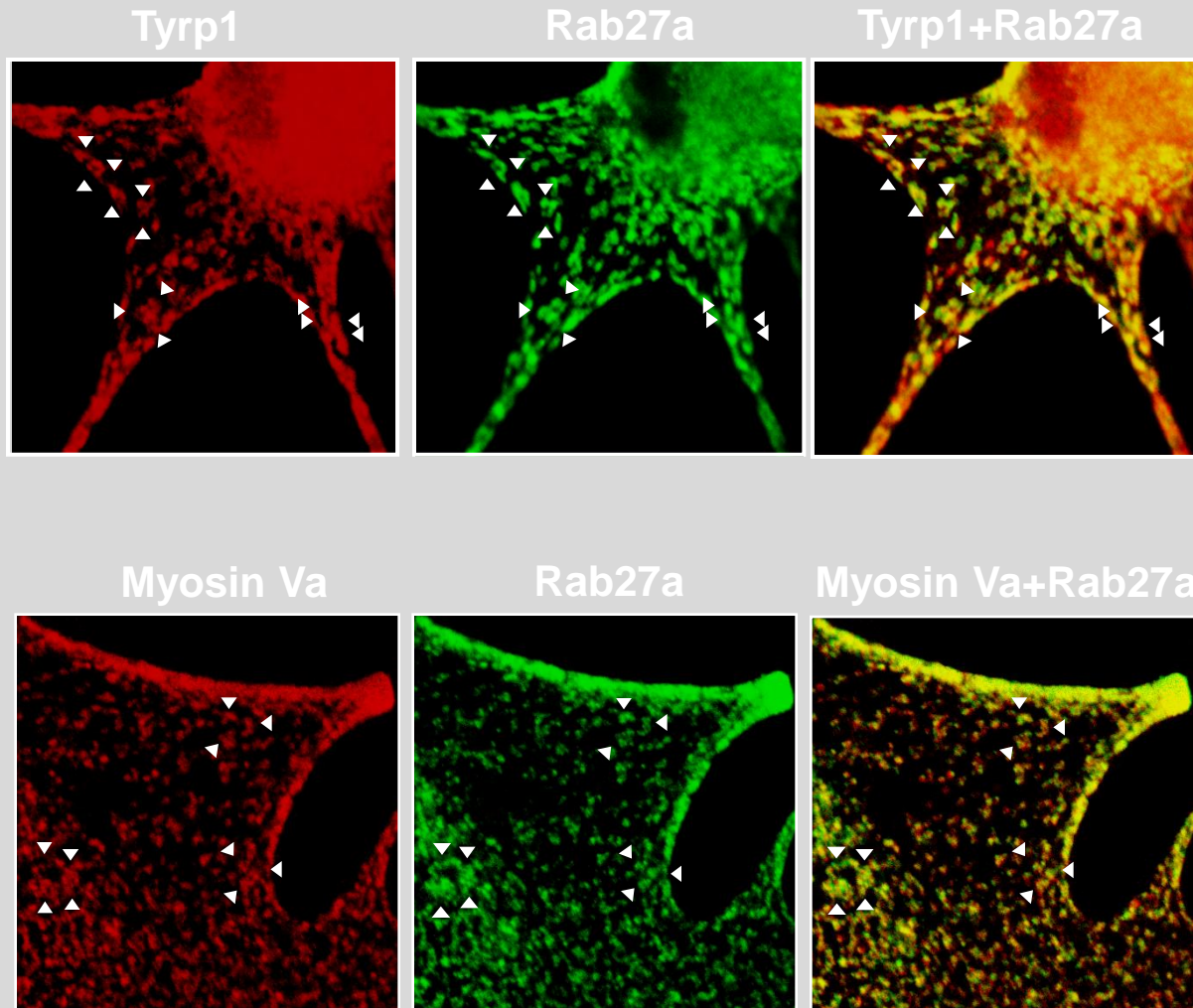


Rab 27a est une molécule-clé du transport des mélanosomes.

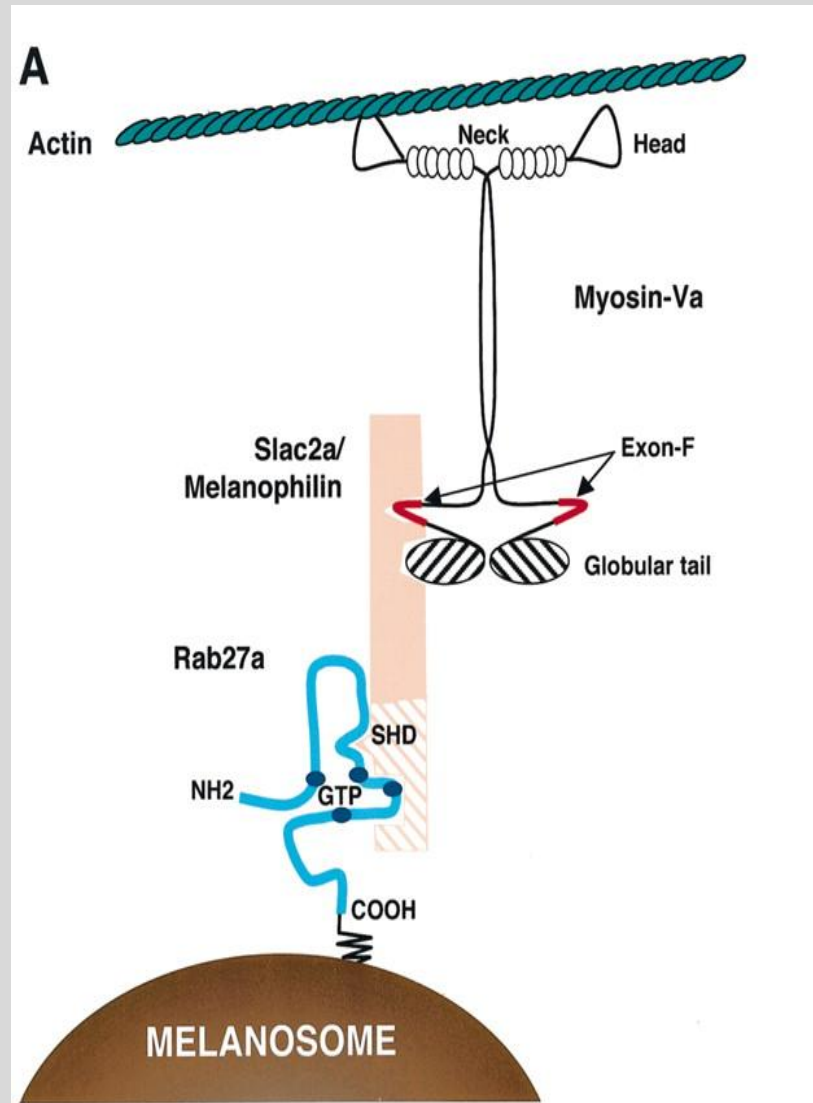


Coopération Rab27a/Myo-Va, dans les mélanocytes ?

Rab27a co-localise avec les mélanosomes et la Myosine Va



Transport des mélanosomes



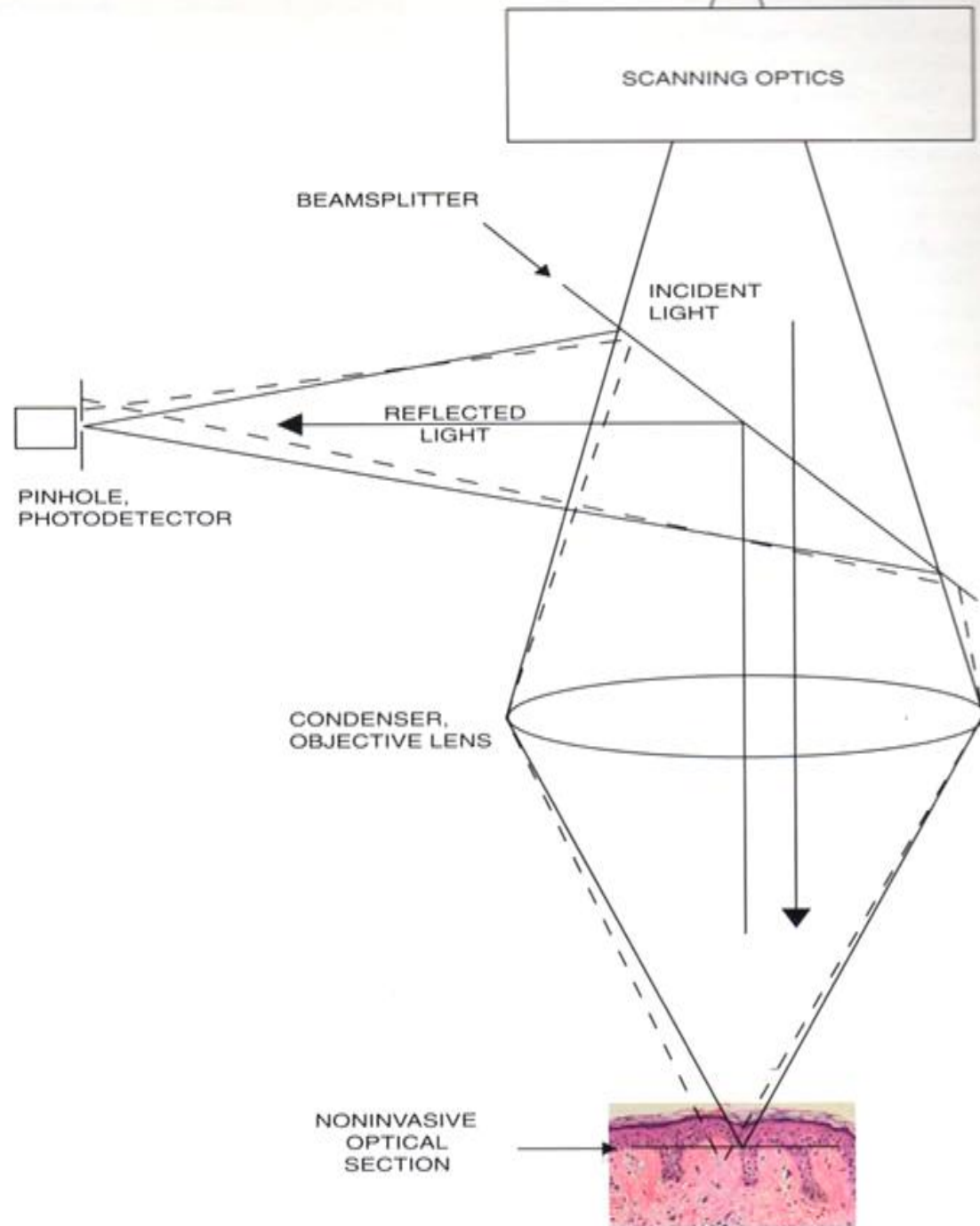
Microscope Confocal *in vivo*



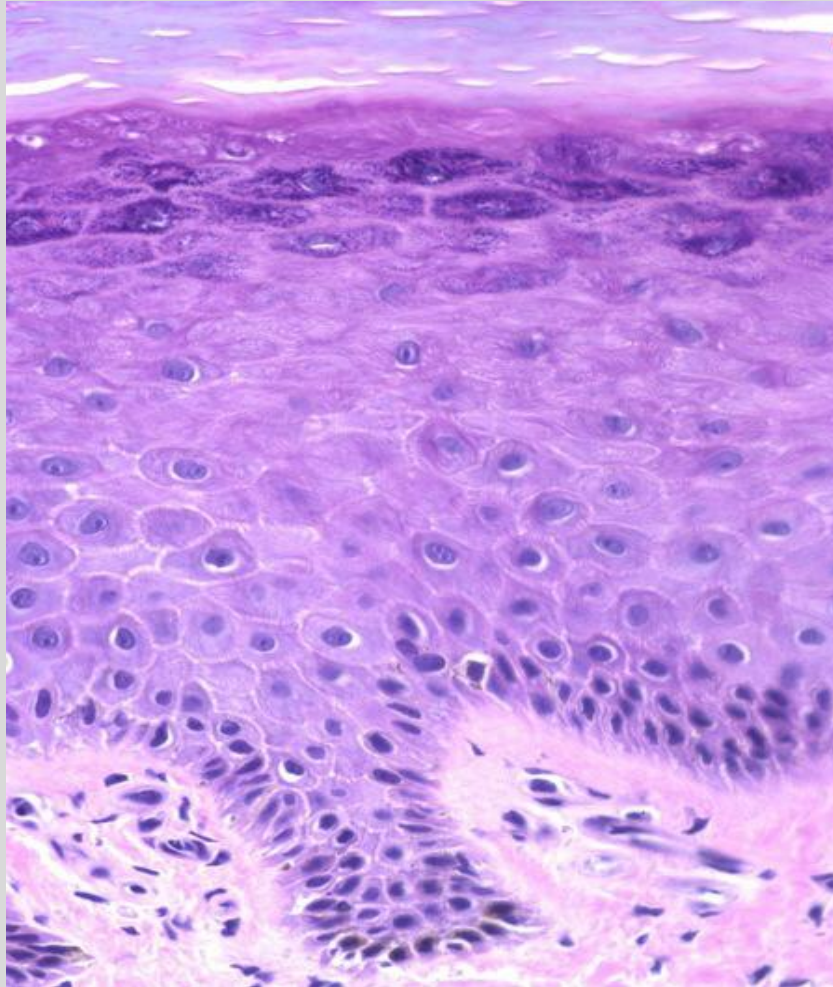
Vivascope 1500

Microscope Confocal *in vivo*

- 830-nm laser source
- power <35 mW/cm²
- lateral resolution of 1.0 mcm
- axial resolution of 3.0 mcm
- imaging depth of 200 -250 mcm



Histologie cutanee



← **Stratum Corneum**


← **Granular layer**
(contains purple keratinohyaline granules)

← **Stratum spinosum**
(spiny processes separate keratinocytes)

← **Basal layer**

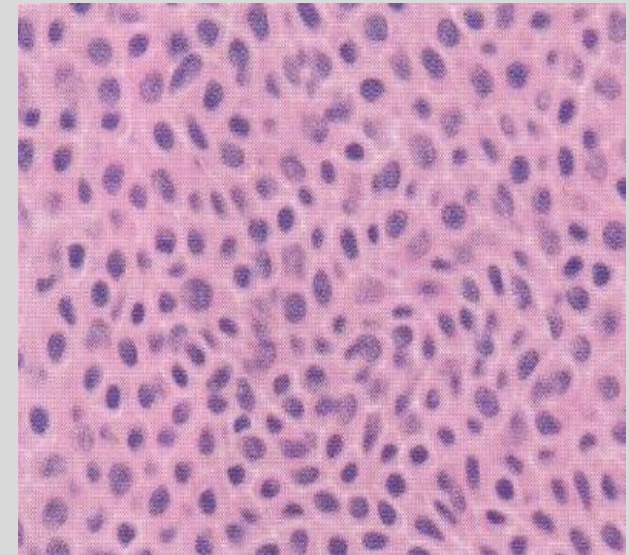
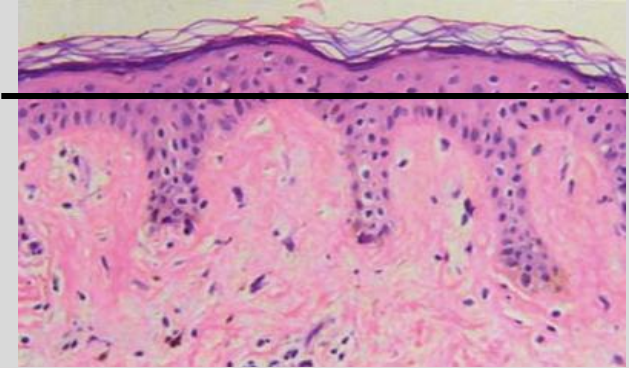
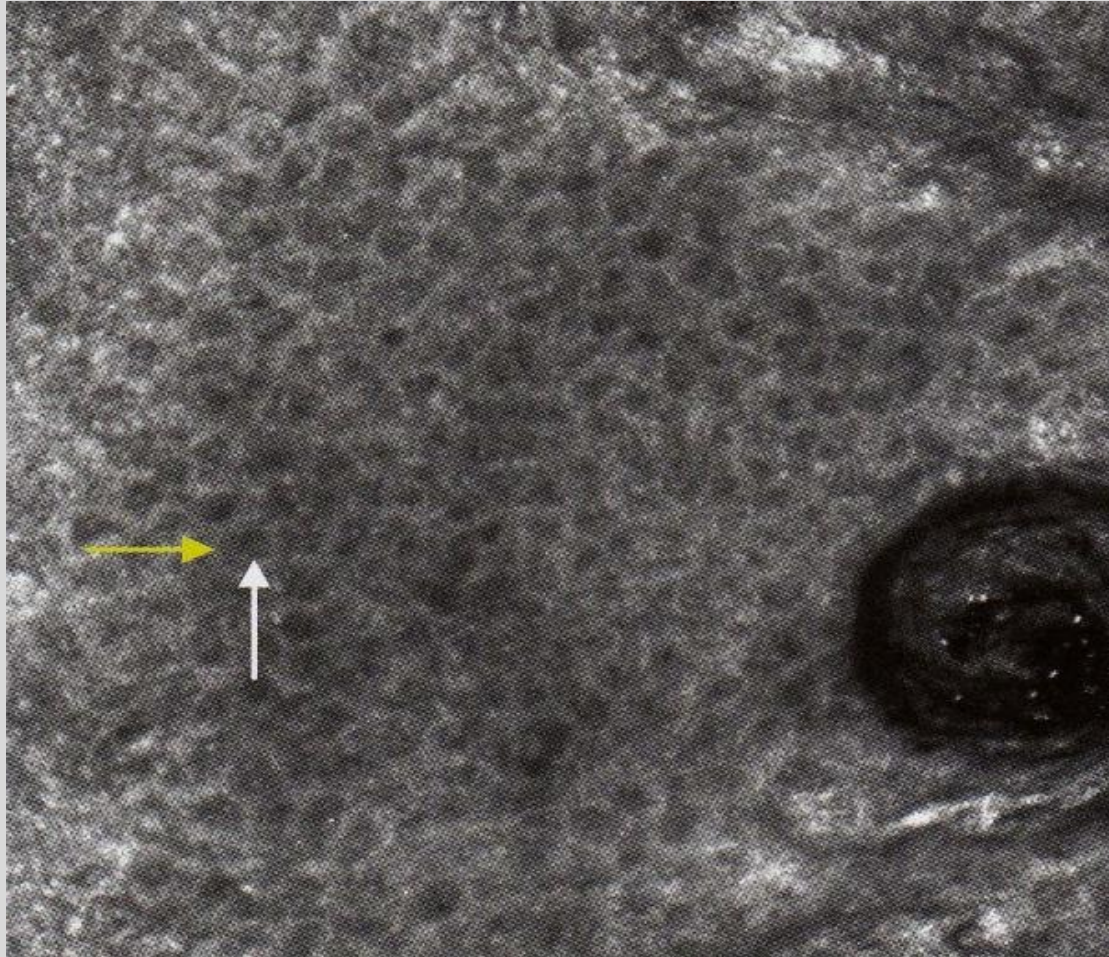
Technique

Table 2.2 Refractile structures in decreasing brightness

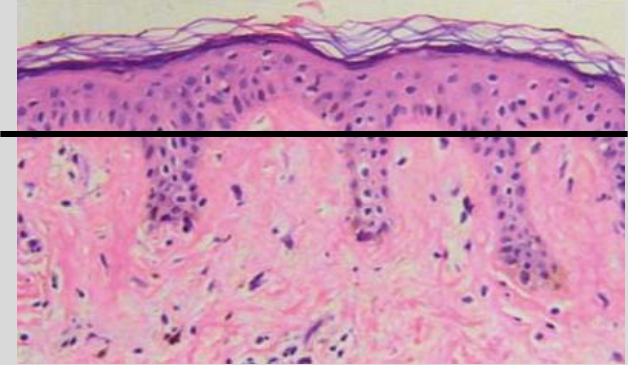
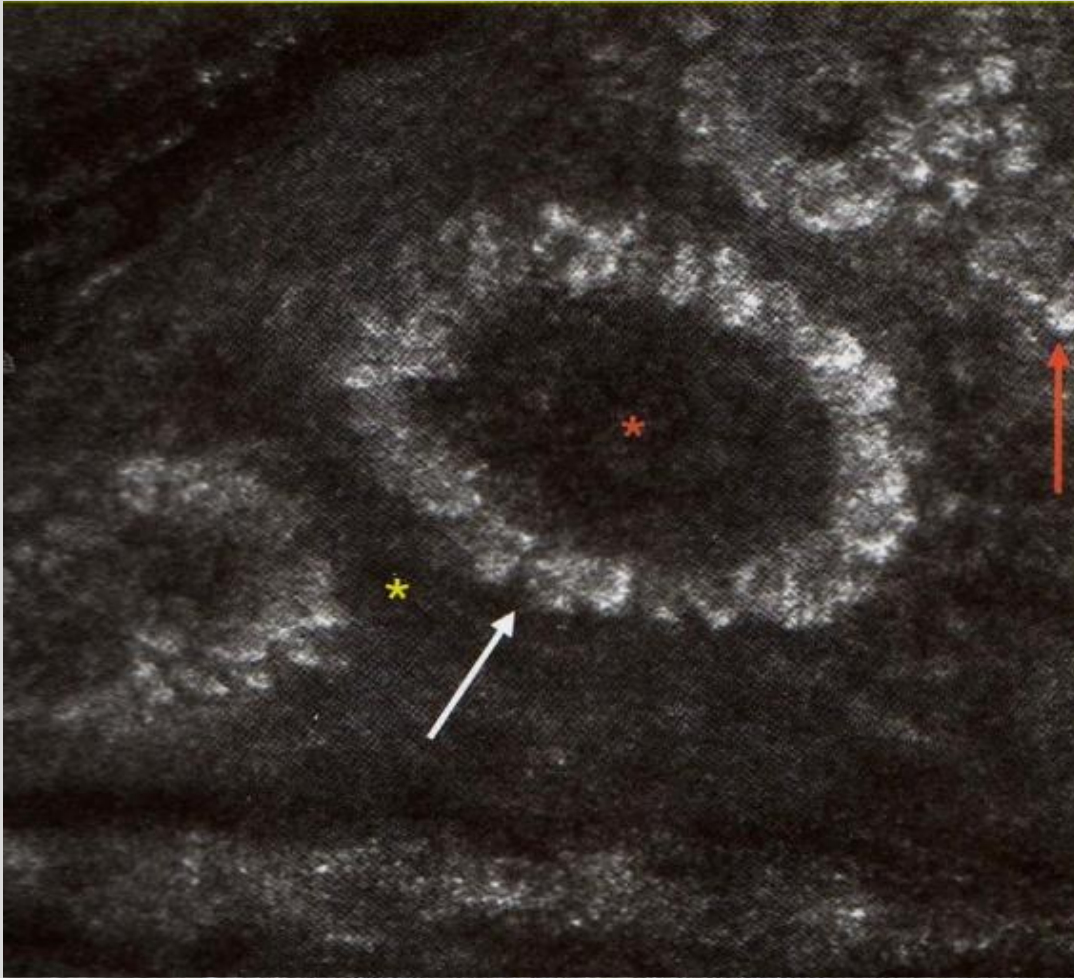
High refractility	Bright
Melanin-containing cells	
Melanocyte cytoplasm	
Melanophage cytoplasm	
Pigmented keratinocyte cytoplasm	
Keratin-containing structures	
Stratum corneum	
Infundibular epithelium	
Hair shaft	
Acrosyringium	
Activated Langerhans cell cytoplasm	
Granulocyte (WBC) cytoplasm	
Medium refractility	
Spinous keratinocyte cytoplasm	
Sebocyte cytoplasm	
Keratohyaline granules	
Nucleoli	
Collagen	
Low refractility	
Red blood cells	
Lymphocytes	
Skin folds (very low)	
Nuclei (very low)	
No refractility	Dark
Air	
Serum	

Gonzalez et al. Reflectance Confocal
Microscopy of Cutaneous Tumors.
Informa. 2007.

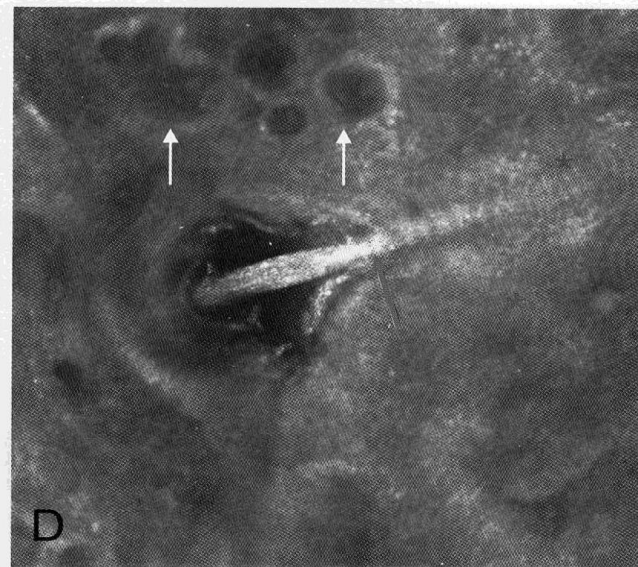
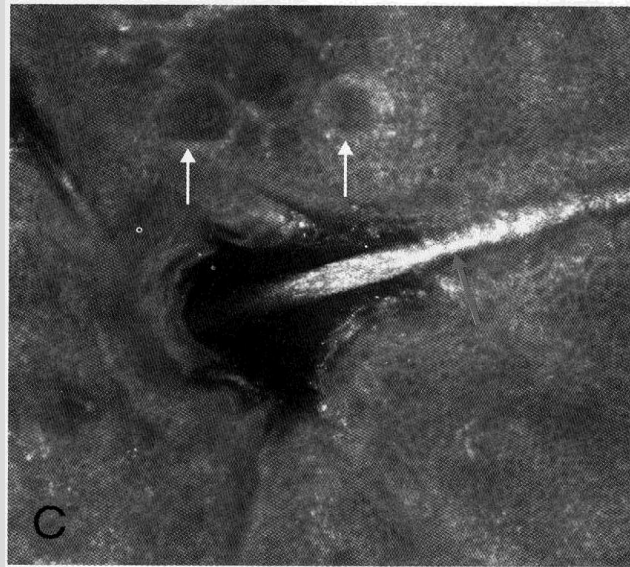
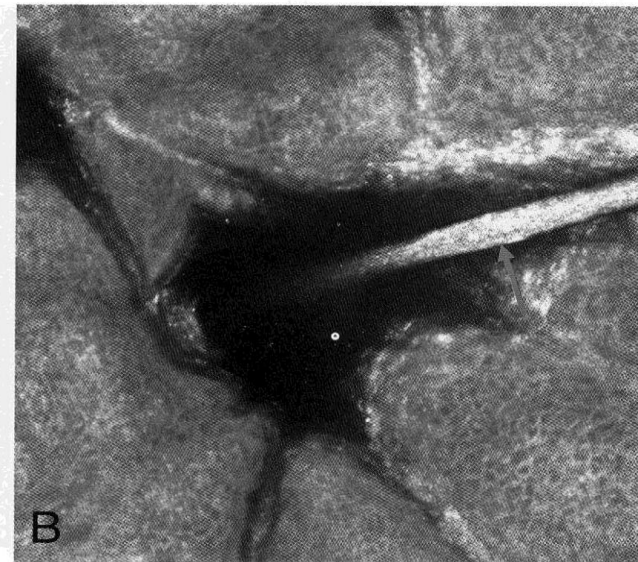
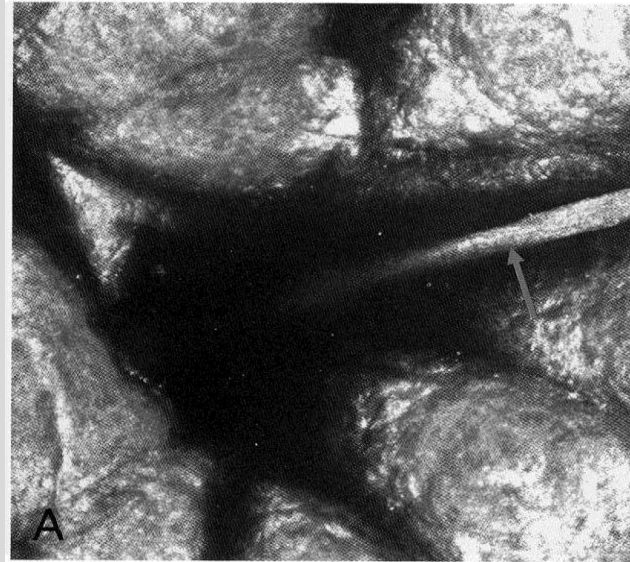
Peau normale: couche spinieuse



Peau normale: couche basale



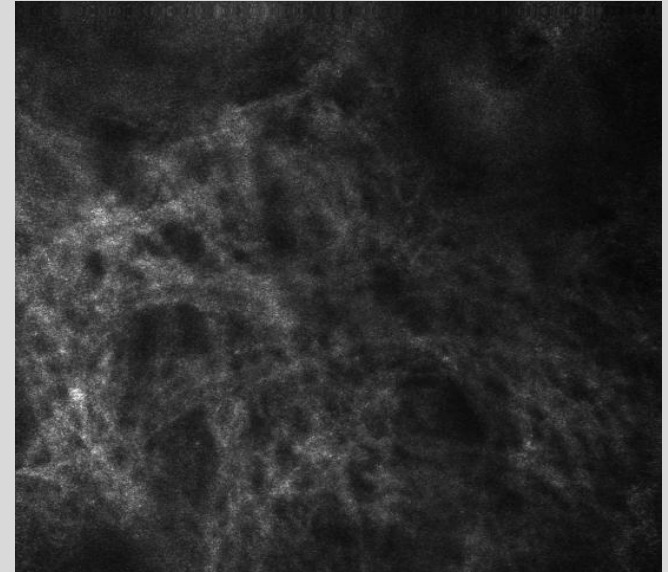
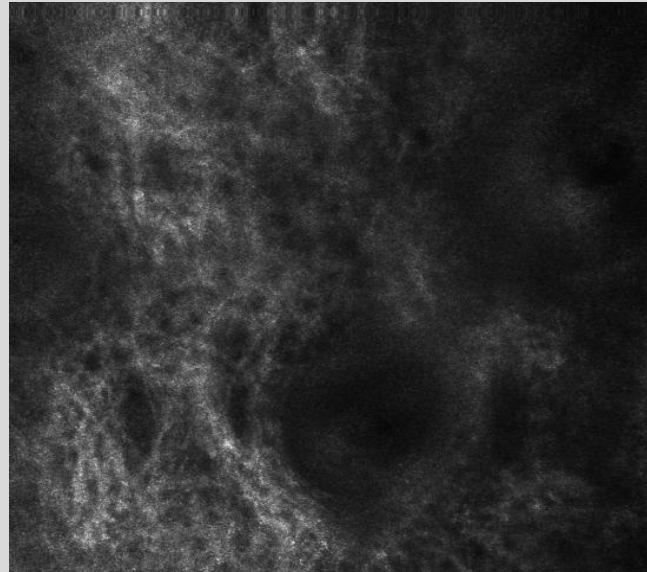
Peau Normale: follicule pileux



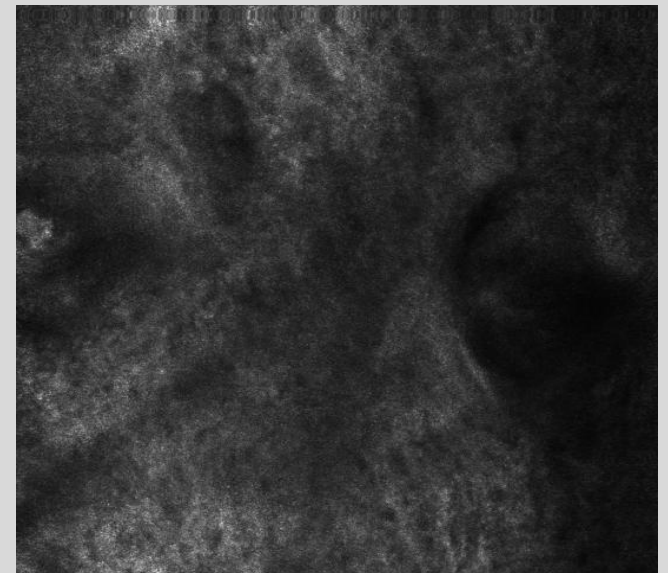
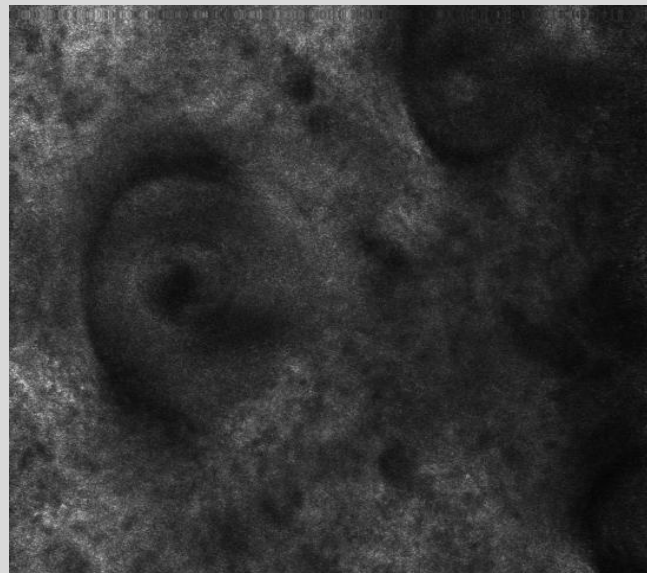
Gonzalez et al. Reflectance Confocal
Microscopy of Cutaneous Tumors.
Informa. 2007.

Fibres de collagène (Vivascope 1500)

Sujet de 22 ans (joue)

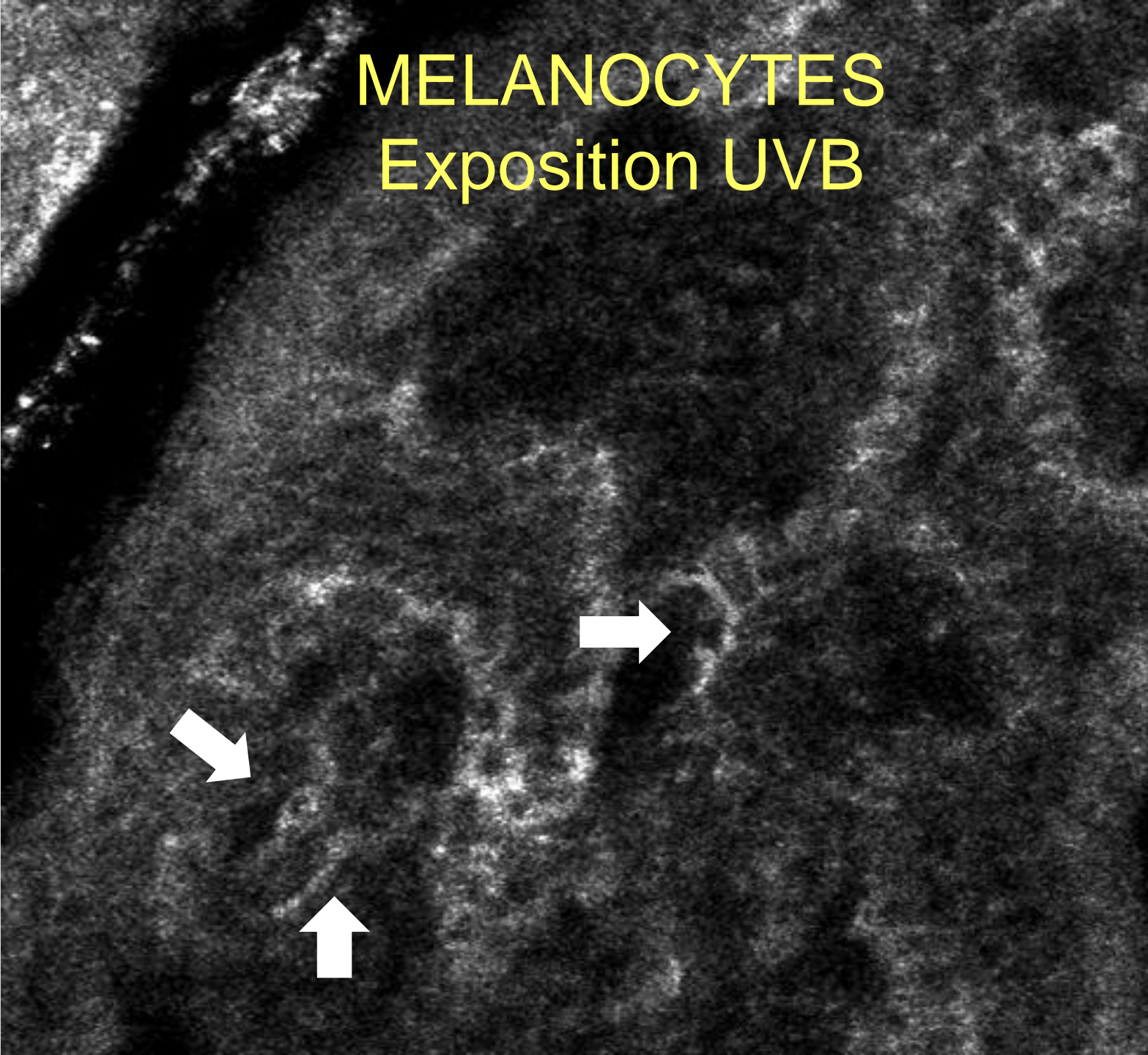


Sujet de 71 ans (joue)

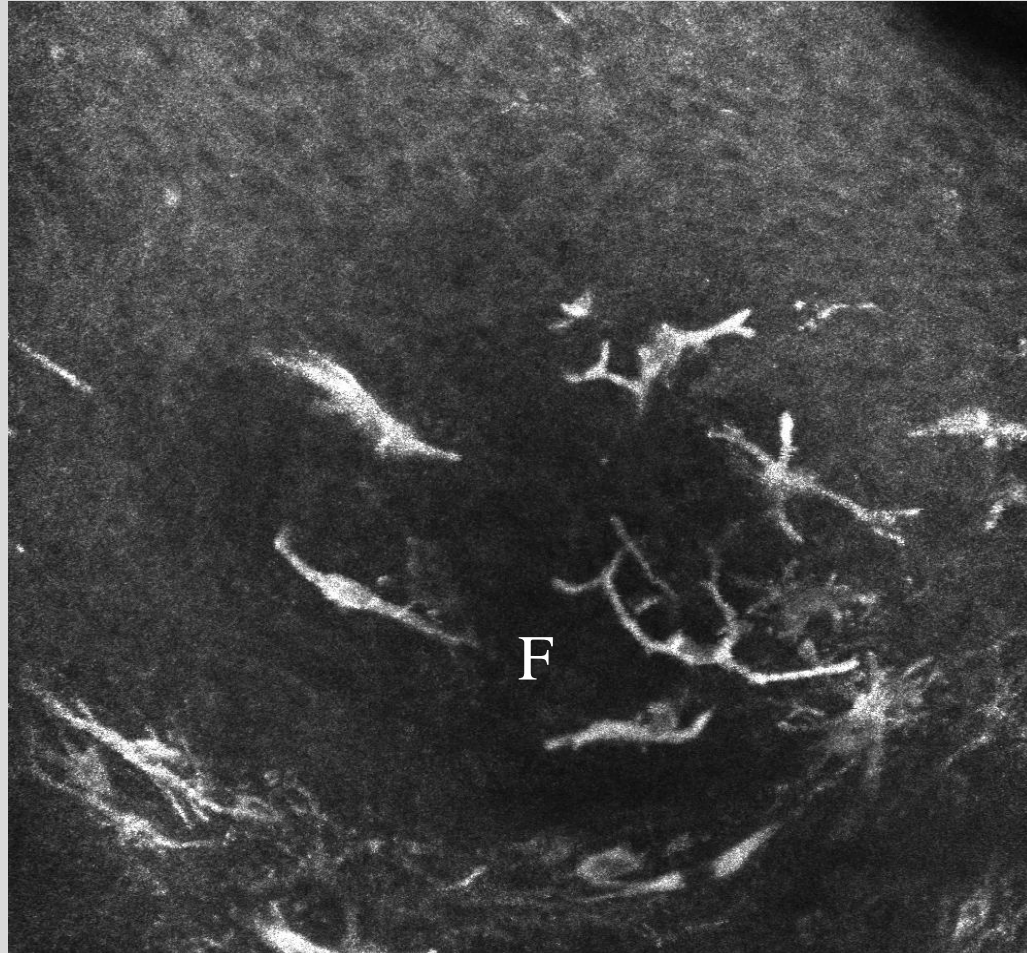


MELANOCYTES

Exposition UVB



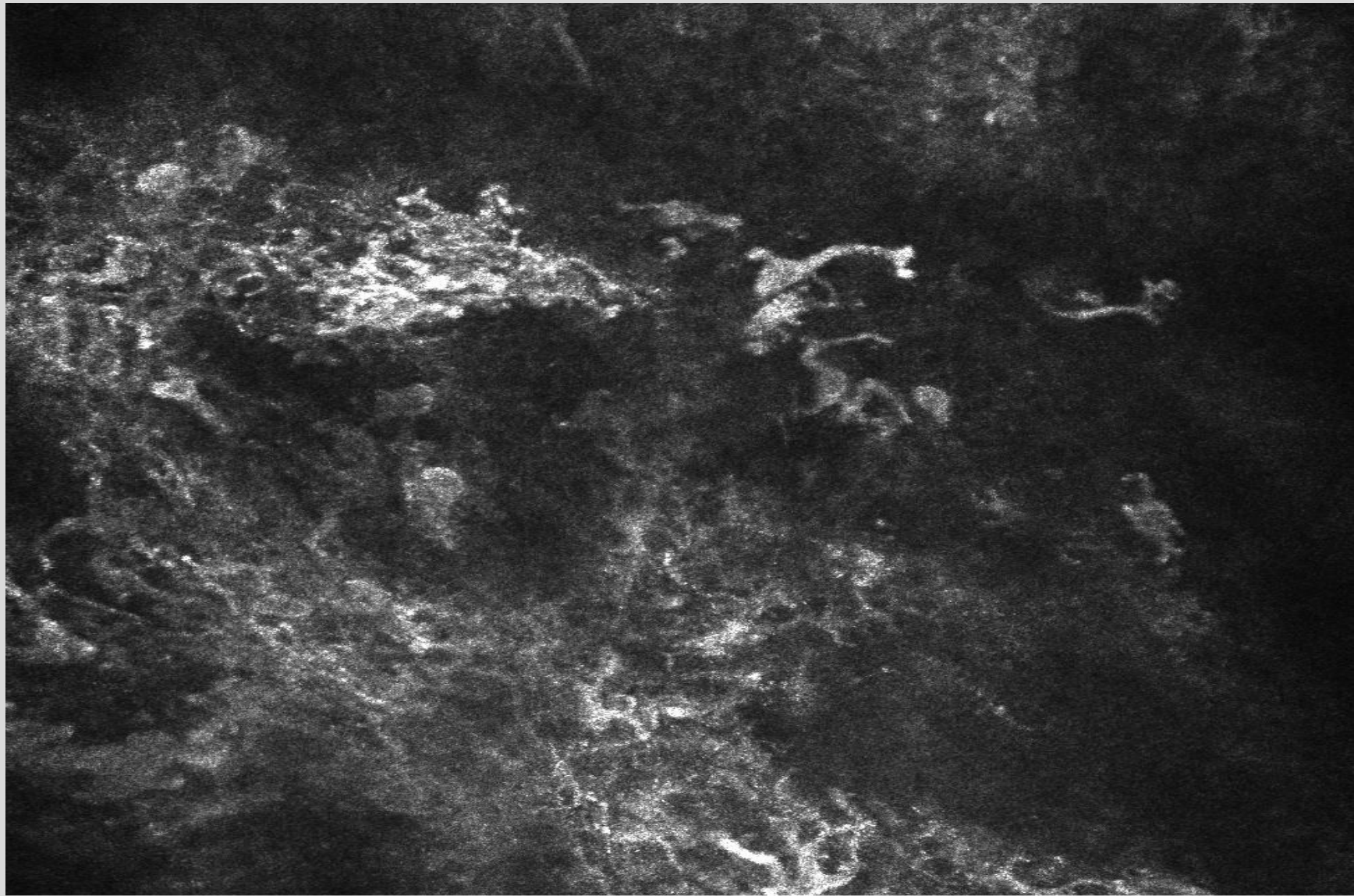
Mélanome / Mélanose de Dubreuilh



cellules atypiques envahissant les follicules pileux

Cas clinique de microscopie confocale





FLUORESCENCE

VivaScope® 1500 Multilaser

Confocal Laser Scanning Microscope for in vivo use with fluorescence (785 nm, 658 nm, 445 nm)

- Combination of reflectance & fluorescence
- Optical biopsy
- Non-invasive
- In real time

The VivaScope 1500 Multilaser combines reflectance with fluorescent confocal laser scanning microscopy. Analog to the standard device VivaScope 1500, skin areas can be viewed in vivo in the infrared range. Wavelengths of 785 nm (near-infrared), 658 nm (red) or 445 nm (blue) are available. Up to three lasers are integrated in one device.



A fluorescent dye (organism-compatible) can be applied to the tissue to be examined prior to using the VivaScope. The respective laser light excites the fluorophore and the emitted fluorescence produces a contrast during the imaging process that may help to display the histological structure due to the dye distribution.

The VivaScope 1500 Multilaser functions can depict differentiated functional tissue aspects dynamically in vivo. Living tissue can be viewed with all wavelengths in sequence.

Standard filters for the following fluorophores are included in the delivery scope: Indocyanine green (near-infrared laser), methylene blue and toluidine blue (red laser), as well as sodium fluorescein (blue laser).

The VivaScope 1500 Multilaser can be combined optimally with the VivaCam®.



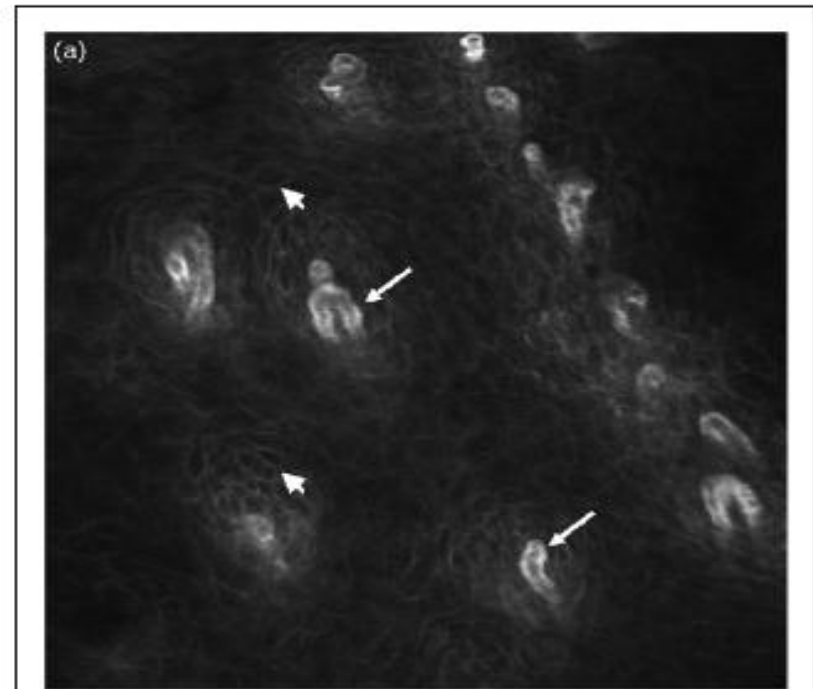
Laserconfiguration VS1500 Multilaser		filter bar positions			
Laser	Application	filter bar No.	Open (Reflectance & Fluorescence)	Low Pass (Reflectance)	High Pass (Fluorescence)
445 nm	Sodium Fluorescein & reflectance	1	none	Passband: 435 – 455 nm	Passband: 468.4 – 668.4 nm
658 nm	Methylene blue & reflectance	2	none	Passband: 640 – 670 nm	Passband: 679.3 – 1497.7 nm
785 nm	Indocyanine green & reflectance	3	none	Passband: 775 – 795 nm	Passband: 803.1 – 1770.7 nm

FLUORESCENCE

Confocal endomicroscopy Kerry Dunbar and Marcia Canto

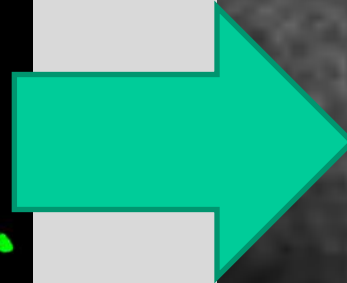
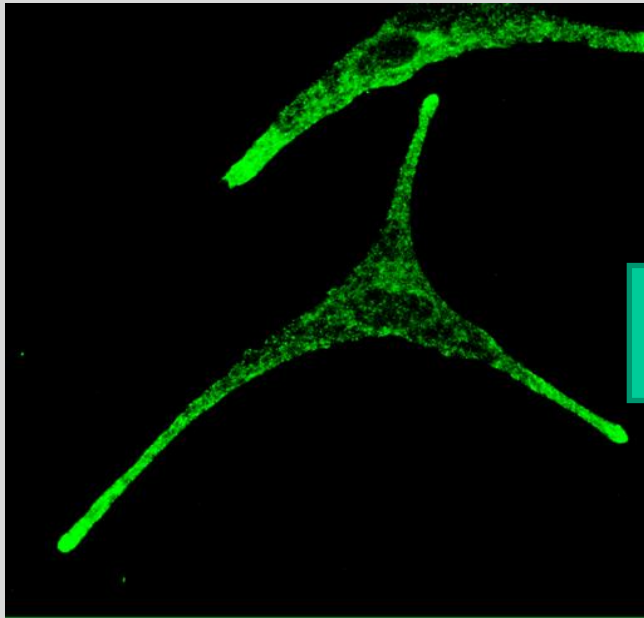
Current Opinion in Gastroenterology 2008,
24:631–637

Figure 1 Confocal endomicroscopy images of the gastrointestinal tract



FITC

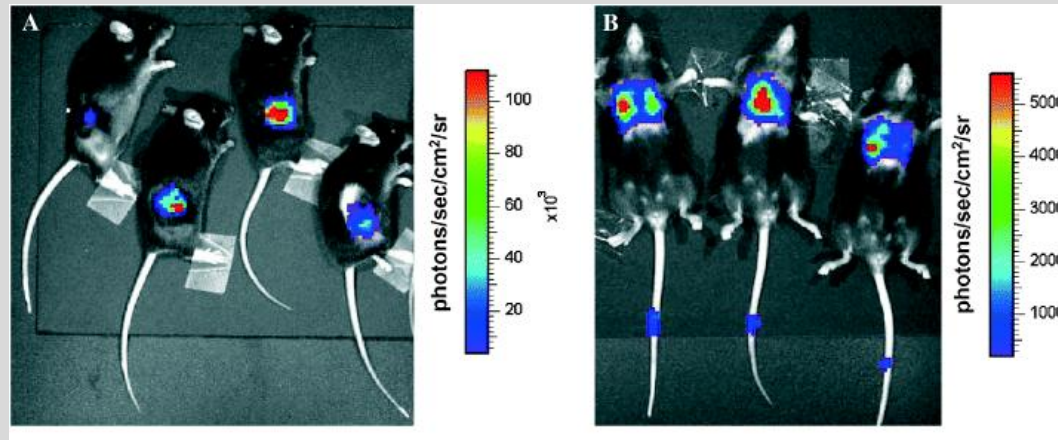
Conclusions



BIOLUMINESCENCE

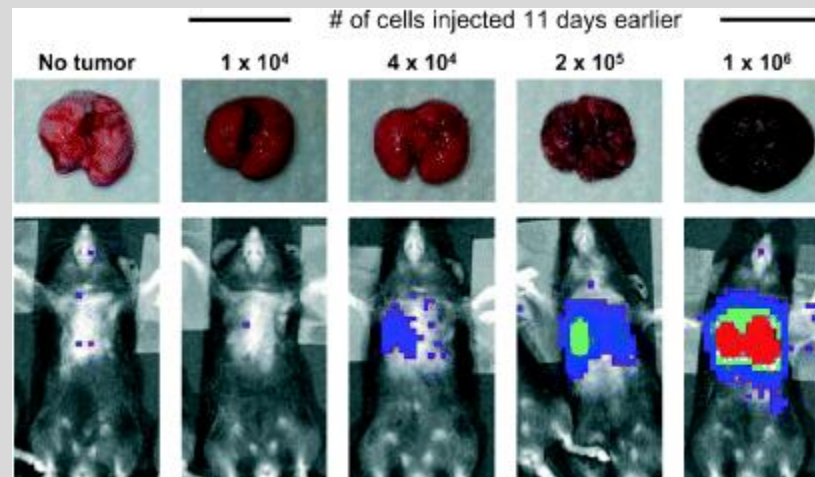
- La **bioluminescence** est la production et l'émission de lumière par un organisme vivant résultant d'une réaction chimique au cours de laquelle l'énergie chimique est convertie en énergie lumineuse.
- Le composé chimique à l'origine de la luminescence est la luciférine. Celle-ci émet de la lumière en s'oxydant grâce à l'intervention de la luciférase, une enzyme.

Imagerie bioluminescente du melanome



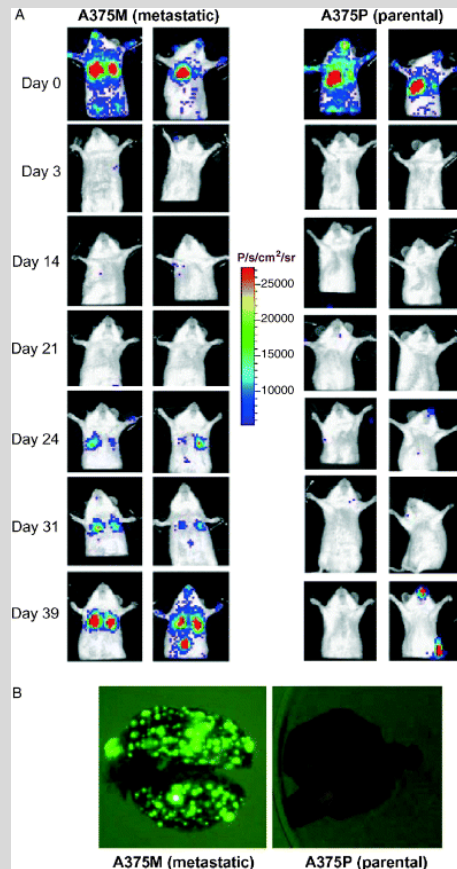
Journal of Investigative Dermatology (2005) **125**, 159–165

Imagerie bioluminescente du melanome



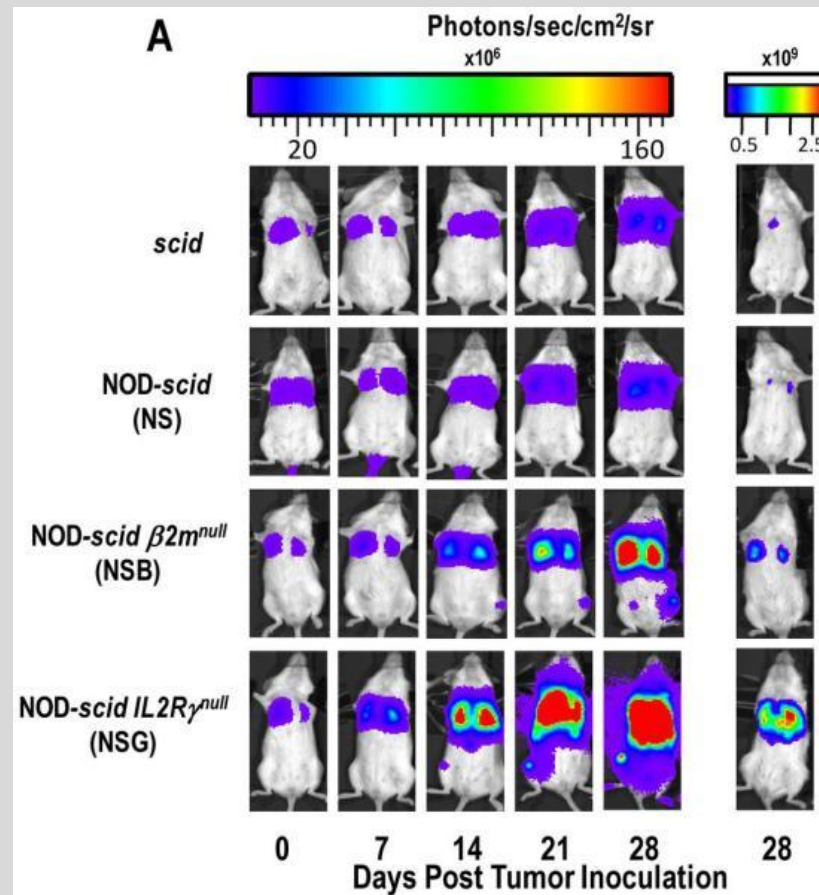
Journal of Investigative Dermatology (2005) **125**, 159–165

Imagerie bioluminescente du melanome



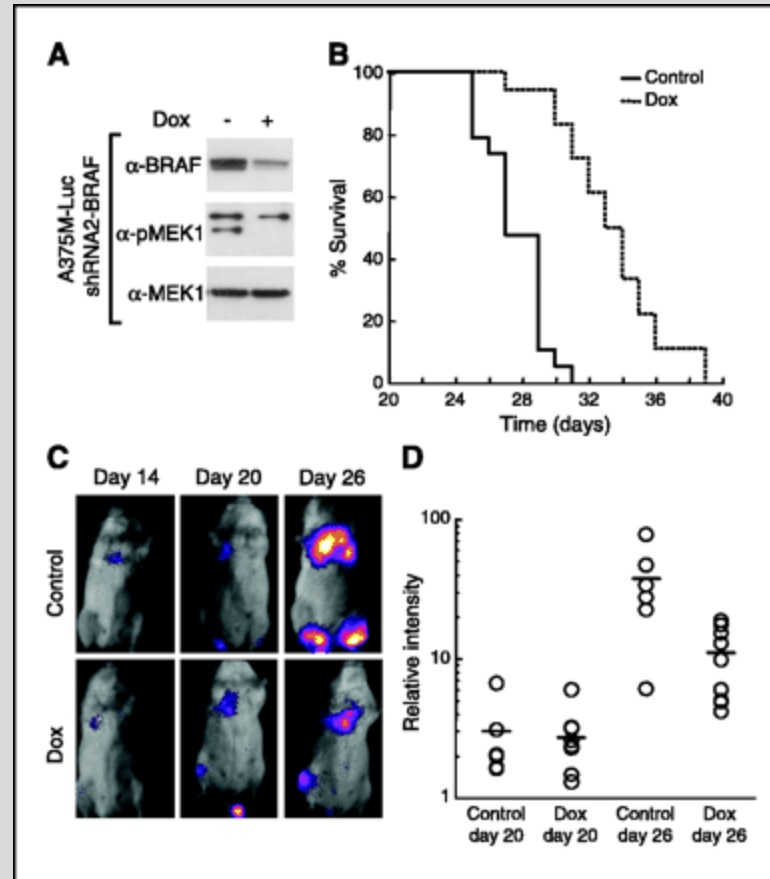
Clin Cancer Res. 2009 May 15;15(10):3277-86.

Imagerie bioluminescente du melanome



Clin Cancer Res. 2009 May 15;15(10):3277-86.

Imagerie bioluminescente du melanome



Cancer Res. 2006 Jan 15;66(2):999-1006.