Studies of Melanocyte Proliferation and Movement

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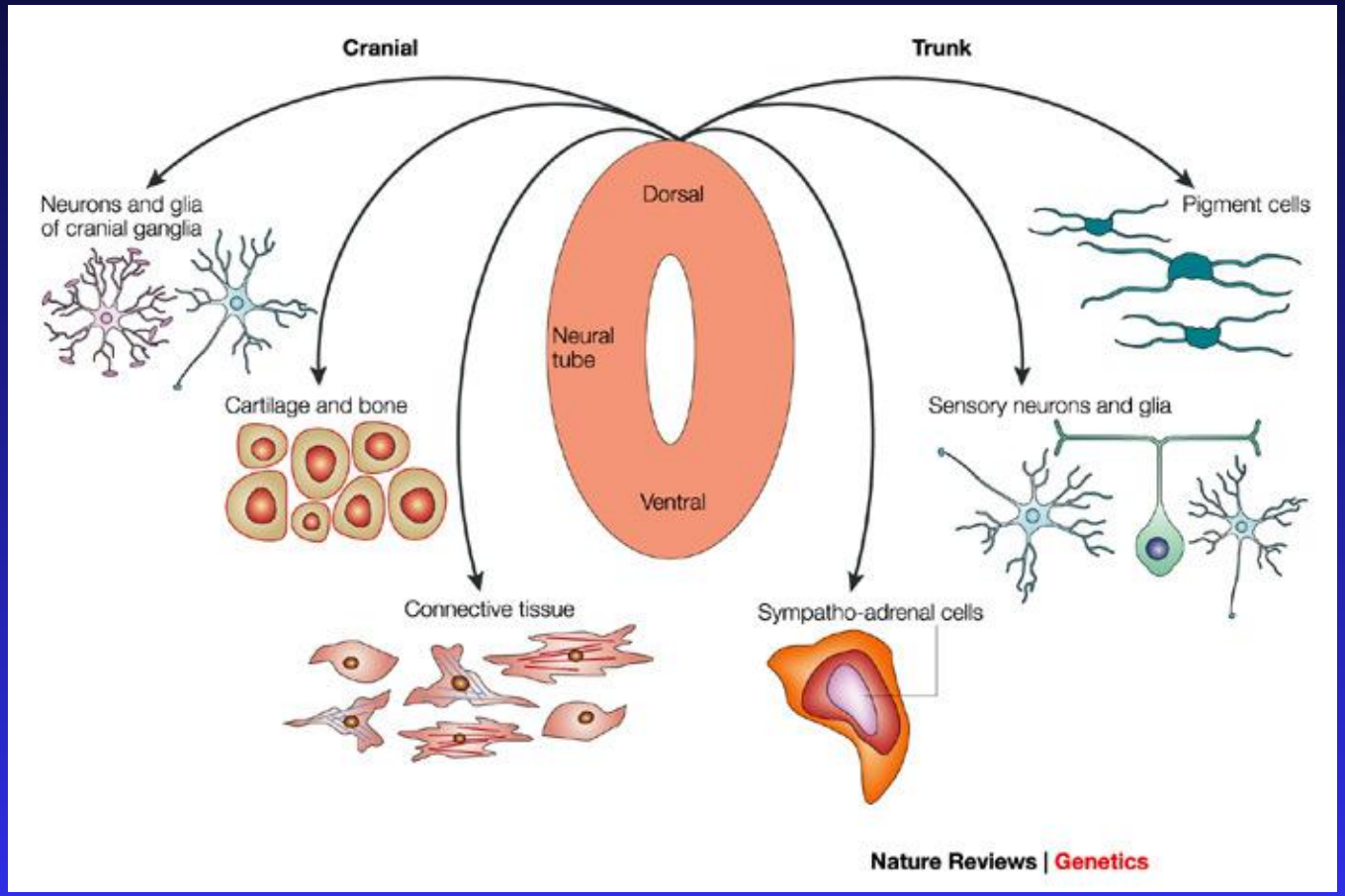
Embryonic origin of melanocytes

- Neural crest migration



Knecht AK and Bronner-Fraser M. Induction of the neural crest: a multigene process
Nature Reviews Genetics 3: 453-461 2002

Concept: Melanoctye migration

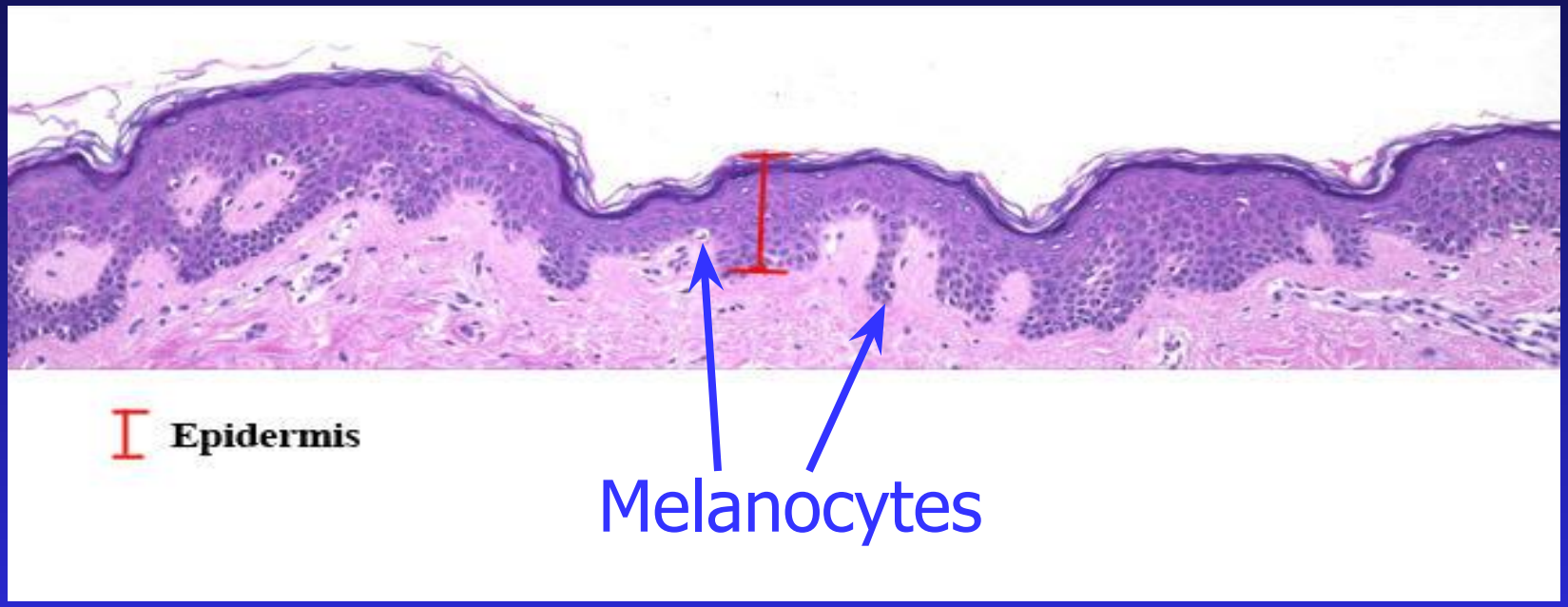
- Intraepidermal--truncal neural crest--ectodermo
- Internal—cardiac neural crest

Concept: Melanoctye migration

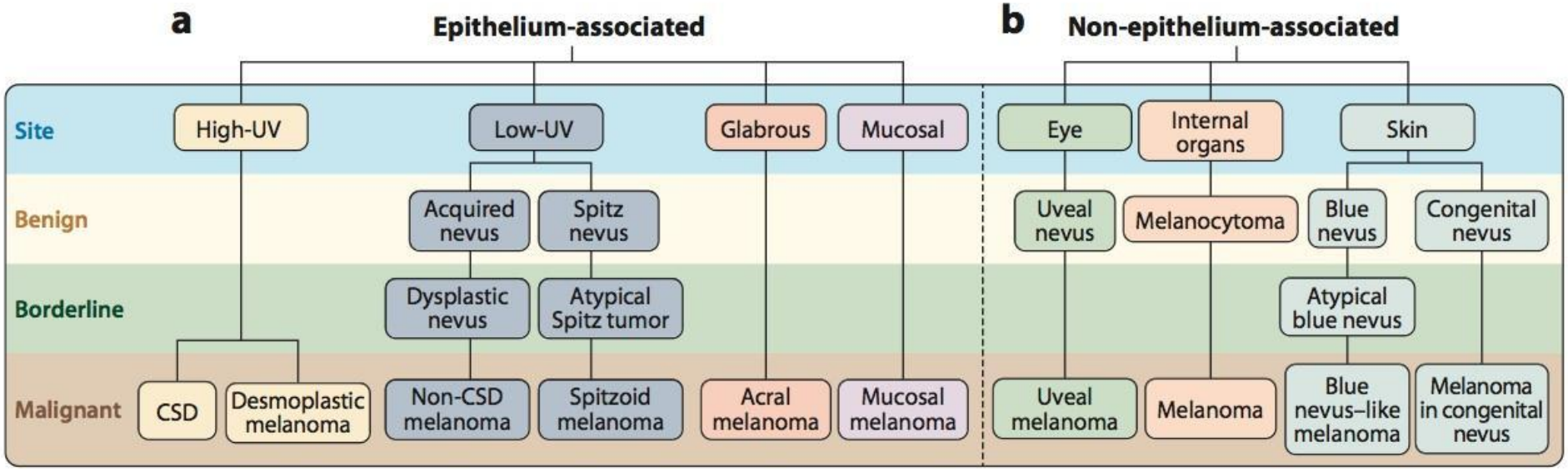
- Intraepidermal--truncal neural crest--ectodermo
- Internal—cardiac neural crest

Cancer=Anaplasia to an embryonic behavior

Intraepidermal melanocytes

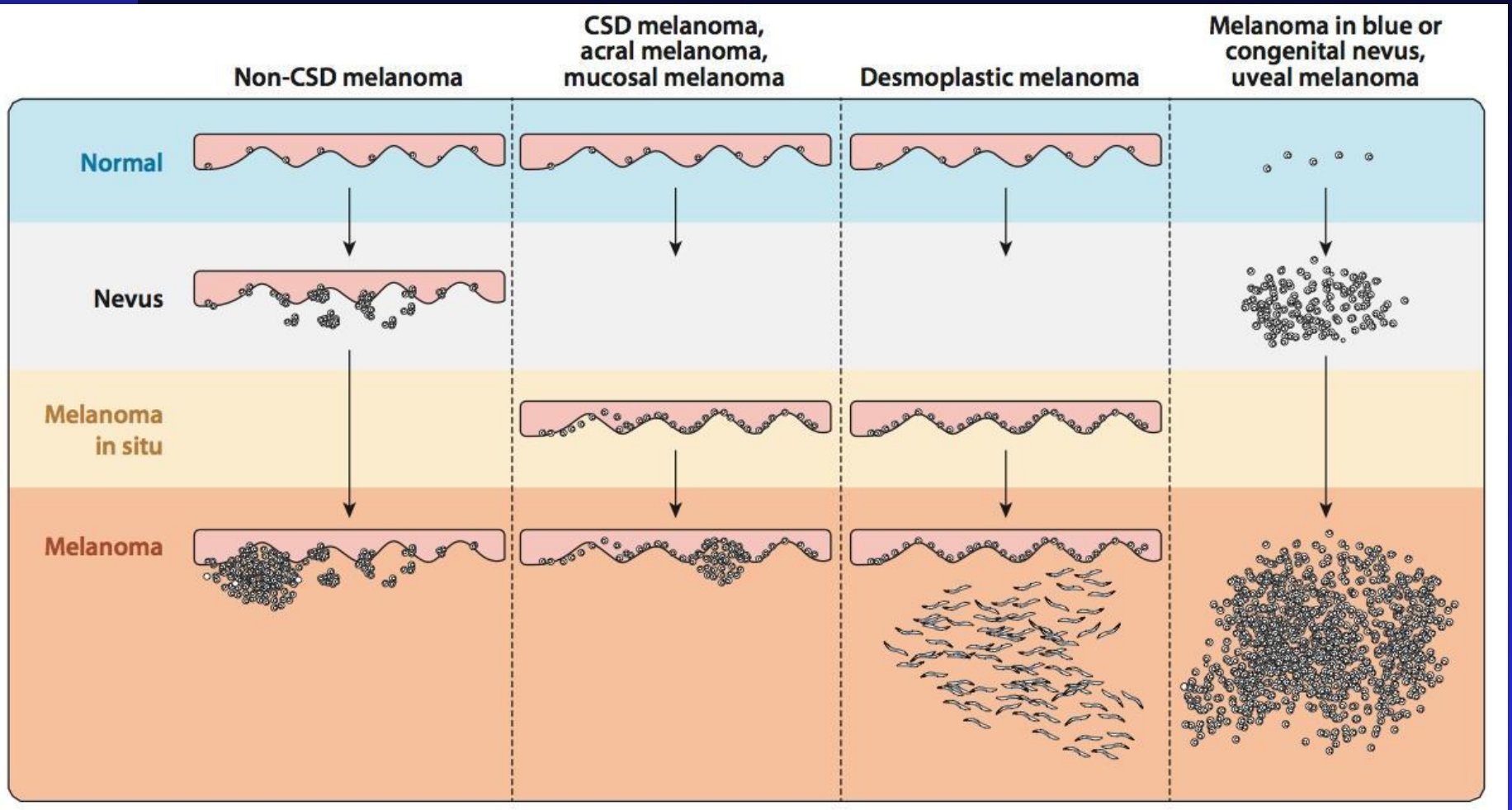


Origin of melanocytic tumors



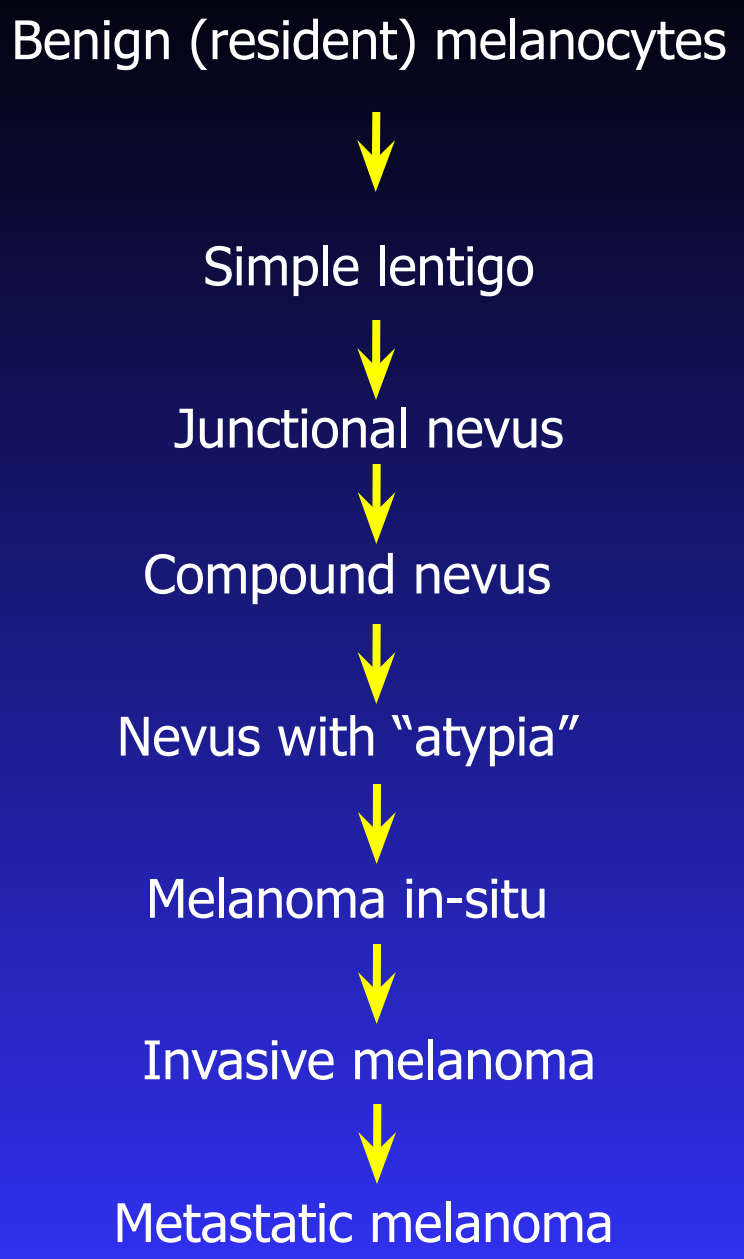
Bastian BC. The molecular pathology of melanoma: an integrated taxonomy of melanocytic neoplasia. Annu Rev Pathol. 2014 Jan 24; 9:239-71.

Ascending and descending melanocytic tumors

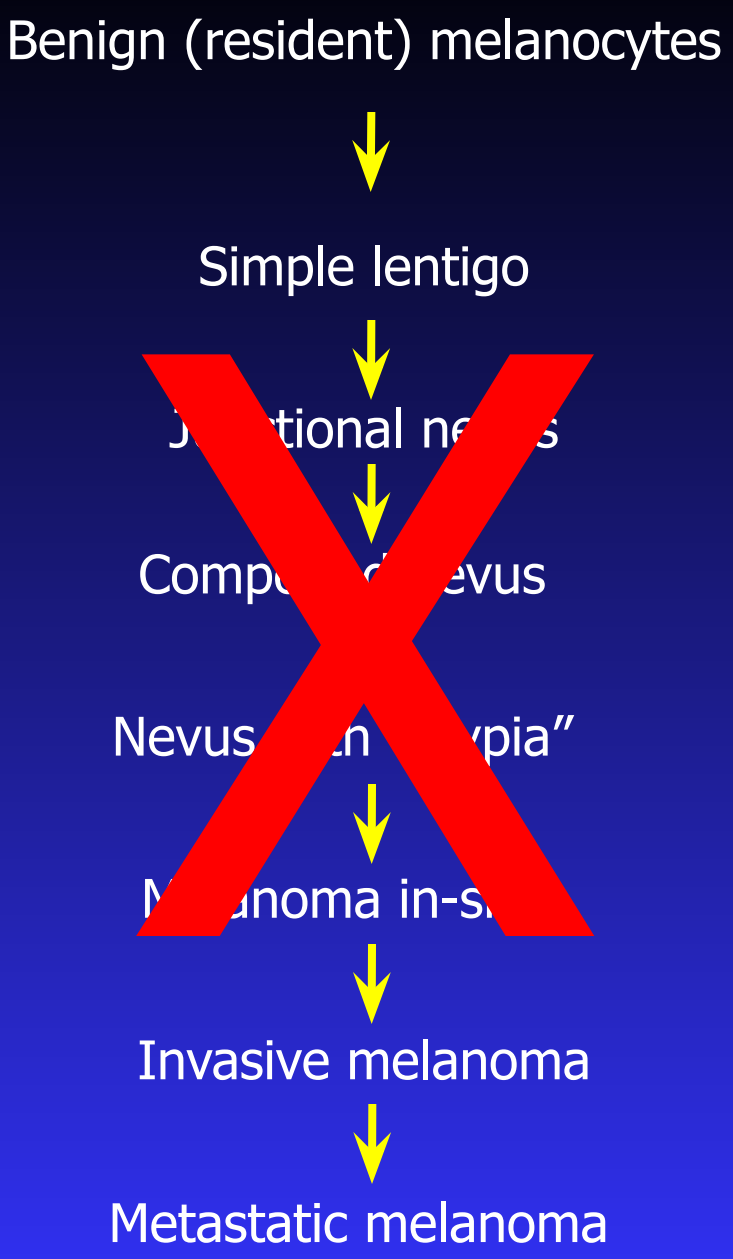


Bastian BC. The molecular pathology of melanoma: an integrated taxonomy of melanocytic neoplasia. Annu Rev Pathol. 2014 Jan 24; 9:239-71.

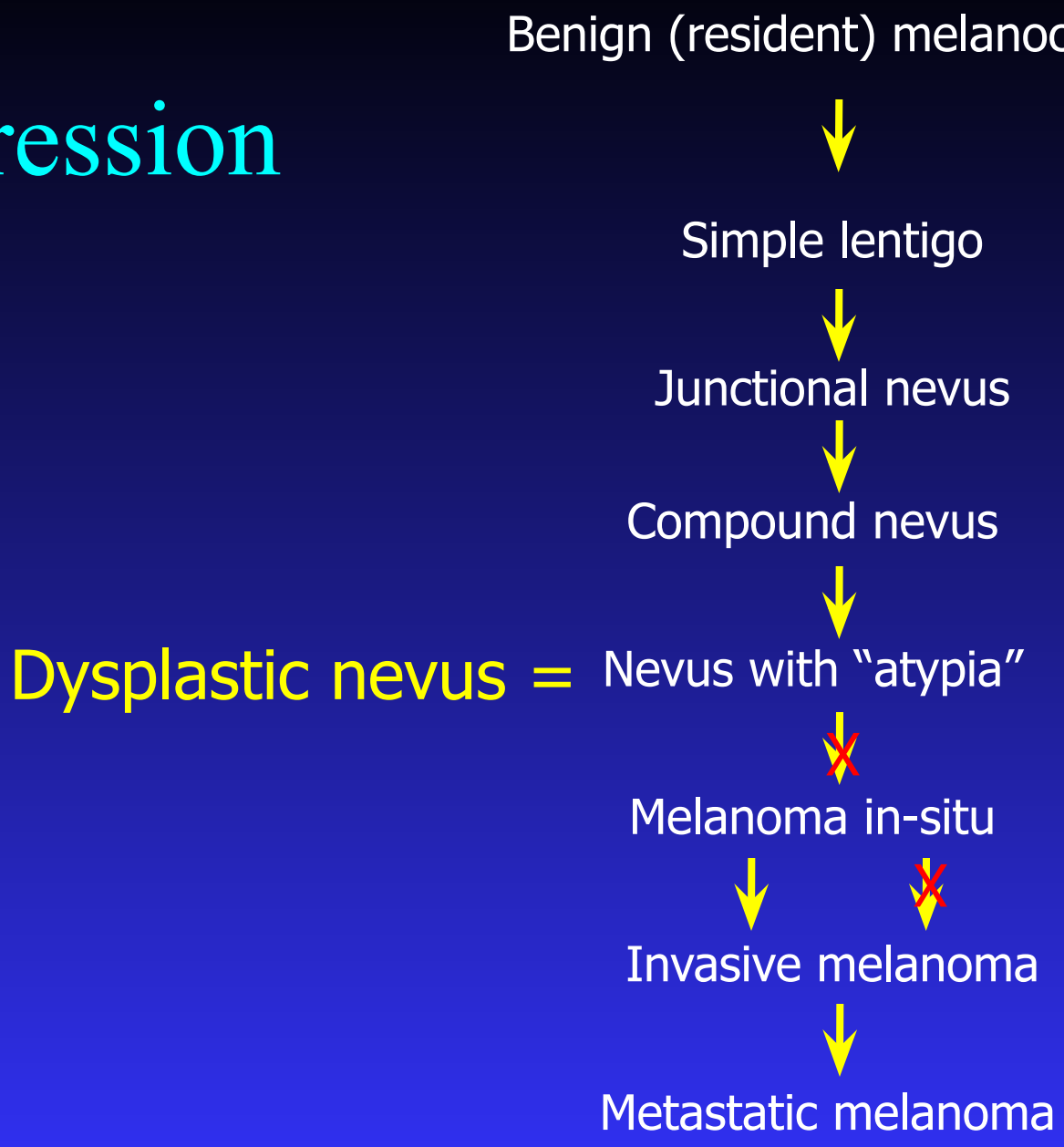
Progression



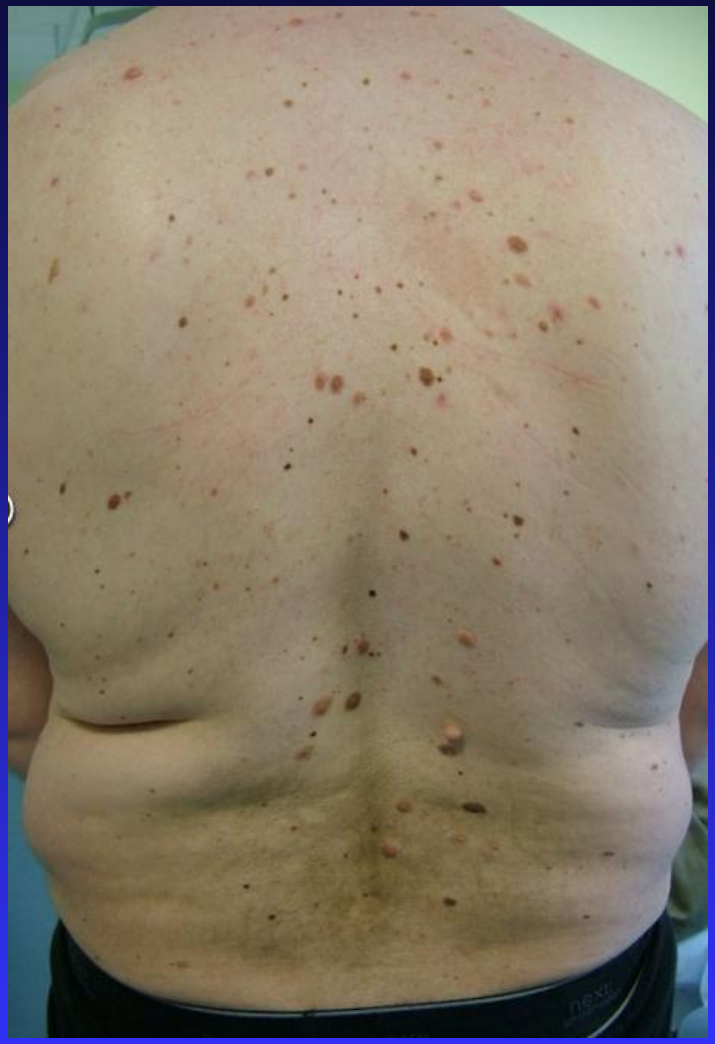
Progression



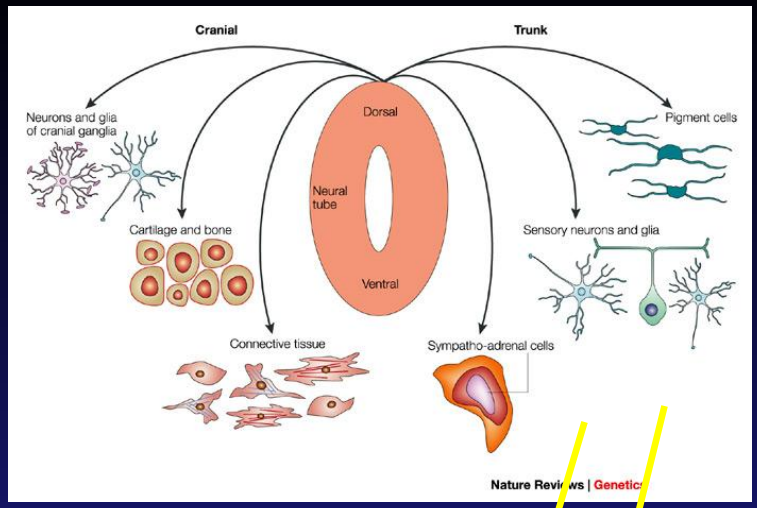
Progression

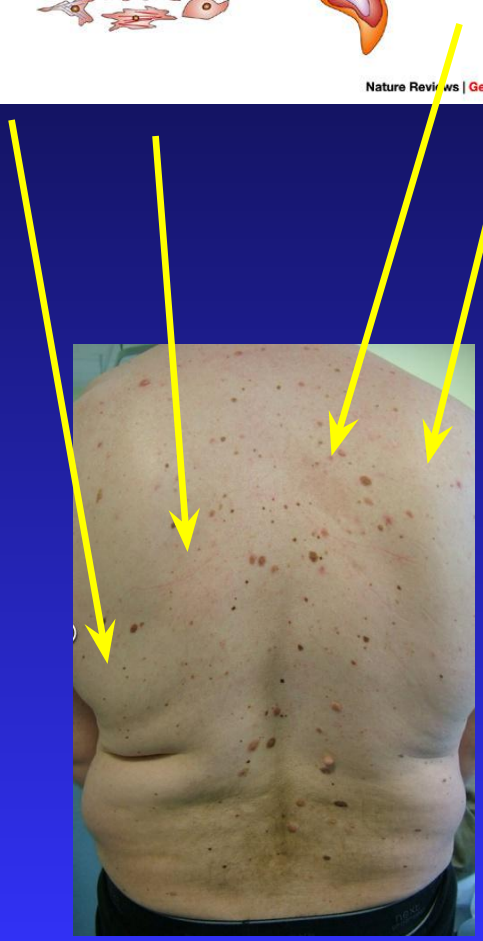


Dysplastic nevus syndrome



Melanomas and atypical nevi





Descendents from a germline mutation?

Fluorescence In Situ Hybridization as an Ancillary Tool in the Diagnosis of Ambiguous Melanocytic Neoplasms

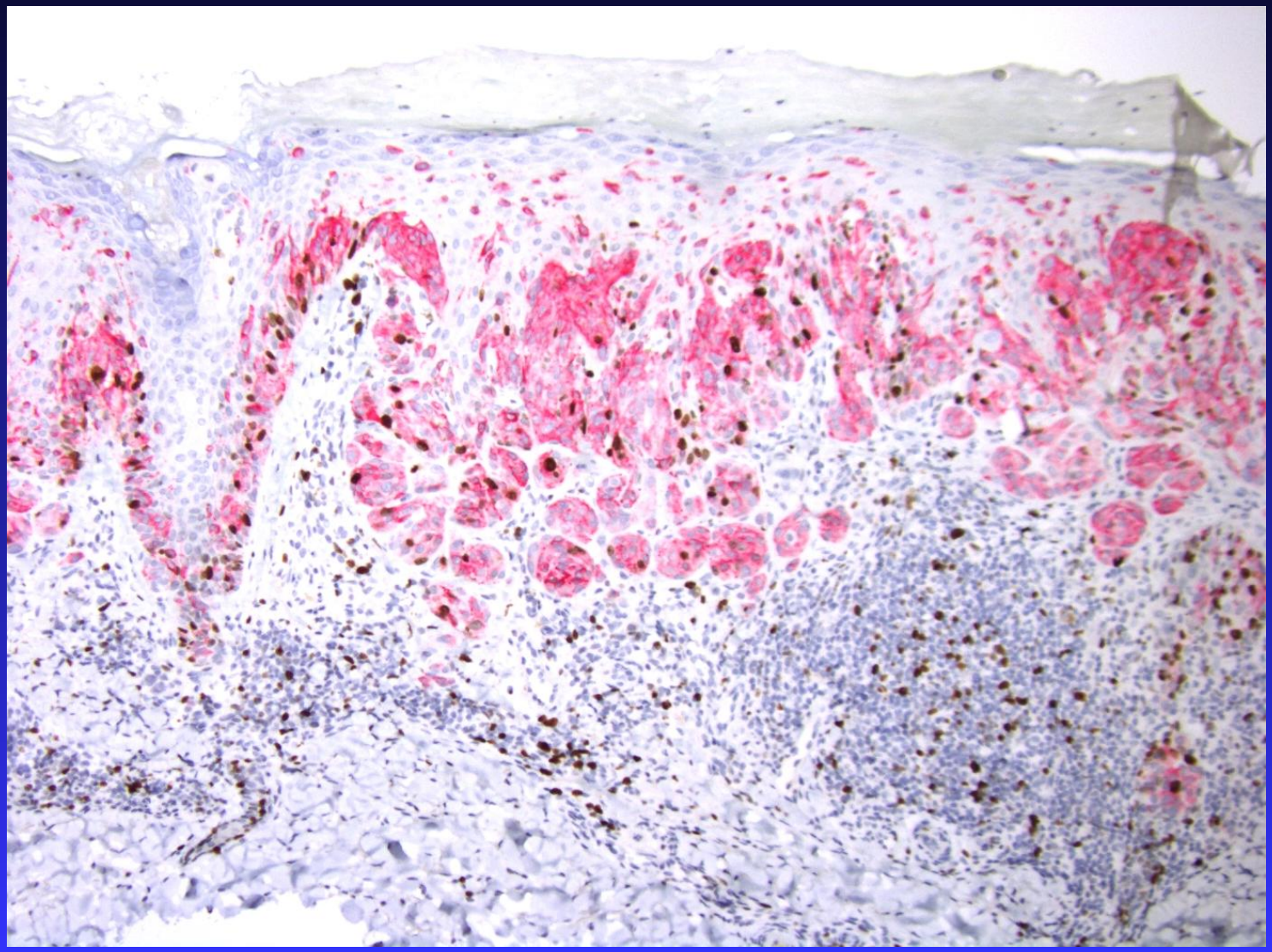
A Review of 804 Cases

Jeffrey P. North, MD,† Maria C. Garrido, MD,‡ Nicholas A. Kolaitis, MD,‡ Philip E. LeBoit, MD,*†‡ Timothy H. McCalmont, MD,*†‡ and Boris C. Bastian, MD*†‡*

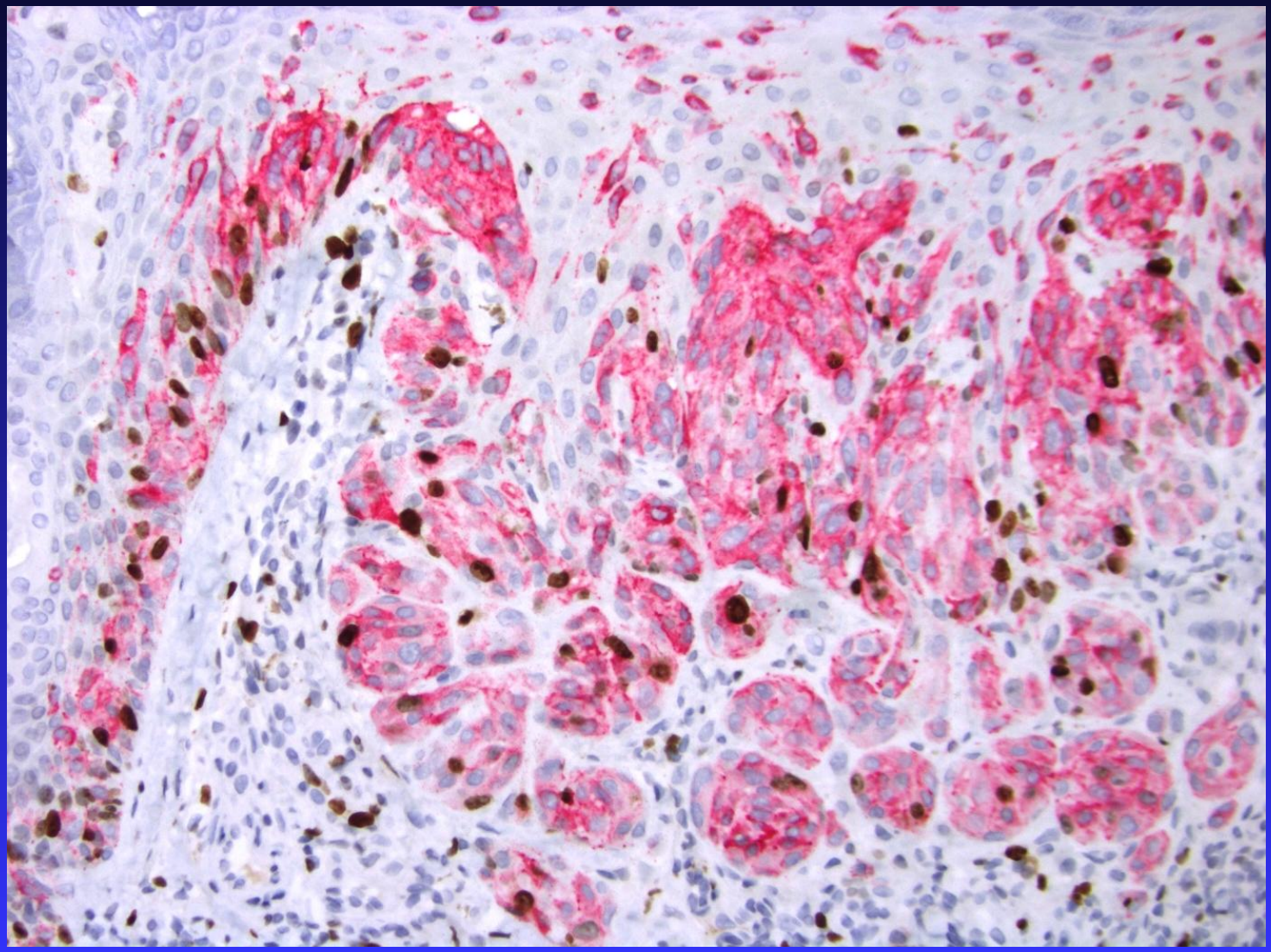
- 630 cases—Negative FISH
  - 489 (78%) benign
  - 91 (14%) ambiguous
  - 50 (8%) malignant

Ki-67/MelanA IHC

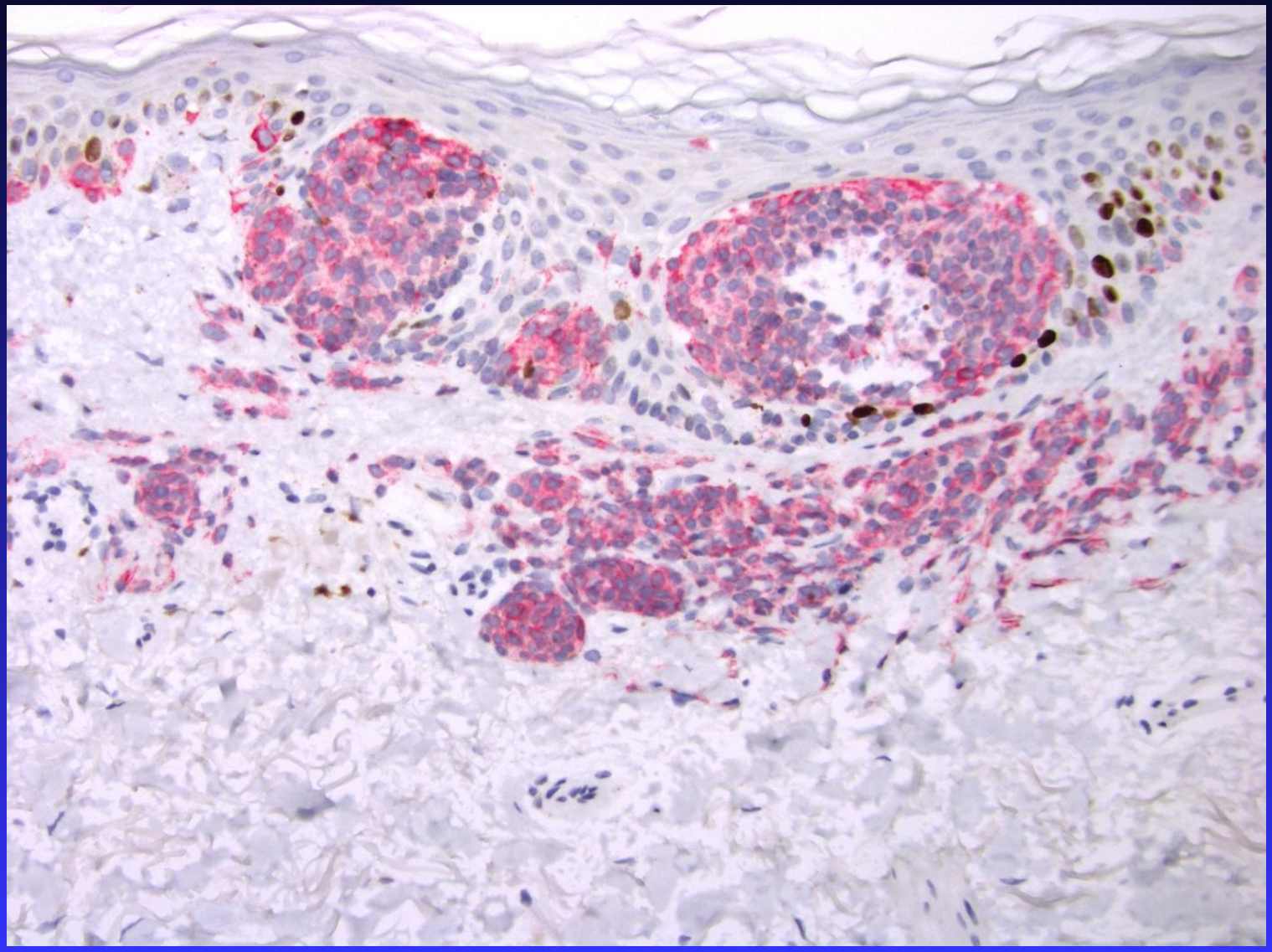
Proliferative rate



Ki-67/MelanA--Melanoma



Ki-67/MelanA—Benign nevus



Quantification of RNA

- Two companies
  - Myriad (myPath)
  - Castle Biosciences (DecisionDX)

Myriad

qRT-PCR Diagnostic Assay

- Expression of the gene signature was analyzed in each of the 50 selected lesions by quantitative reverse transcription polymerase chain reaction (qRT-PCR).
- An expression score was calculated on a scale of -14.9 to +9.6 using a proprietary formula.
  - Scores from 0 to +9.6 were reported as "likely malignant".
  - Scores from -14.9 to -0.1 were reported as "likely benign".

Castle Biosciences (DecisionDx)

Dhillon, et al. Gene expression profile signature (DecisionDx-Melanoma) to predict visceral metastatic risk in patients with Stage I and Stage II cutaneous melanoma. J Clin Oncol 2012;30(suppl; abstr 8543).

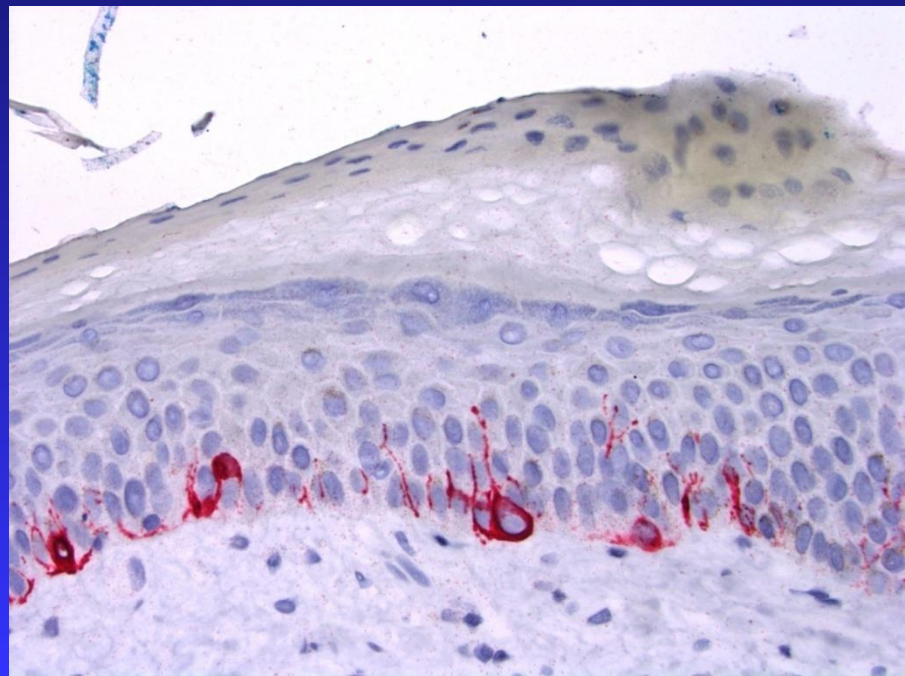
Castle Biosciences (DecisionDx)

Dhillon, et al. Gene expression profile signature (DecisionDx-Melanoma) to predict visceral metastatic risk in patients with Stage I and Stage II cutaneous melanoma. J Clin Oncol 2012;30(suppl; abstr 8543).

DNA Sequencing

Movement of melanocytes in the epidermis

- Normal movement
  - Embryogenesis
  - Repigmentation (vitiligo), recurrent, over a scar
- Melanoma in-situ



Review

Expression and function of cell adhesion molecules during neural crest migration

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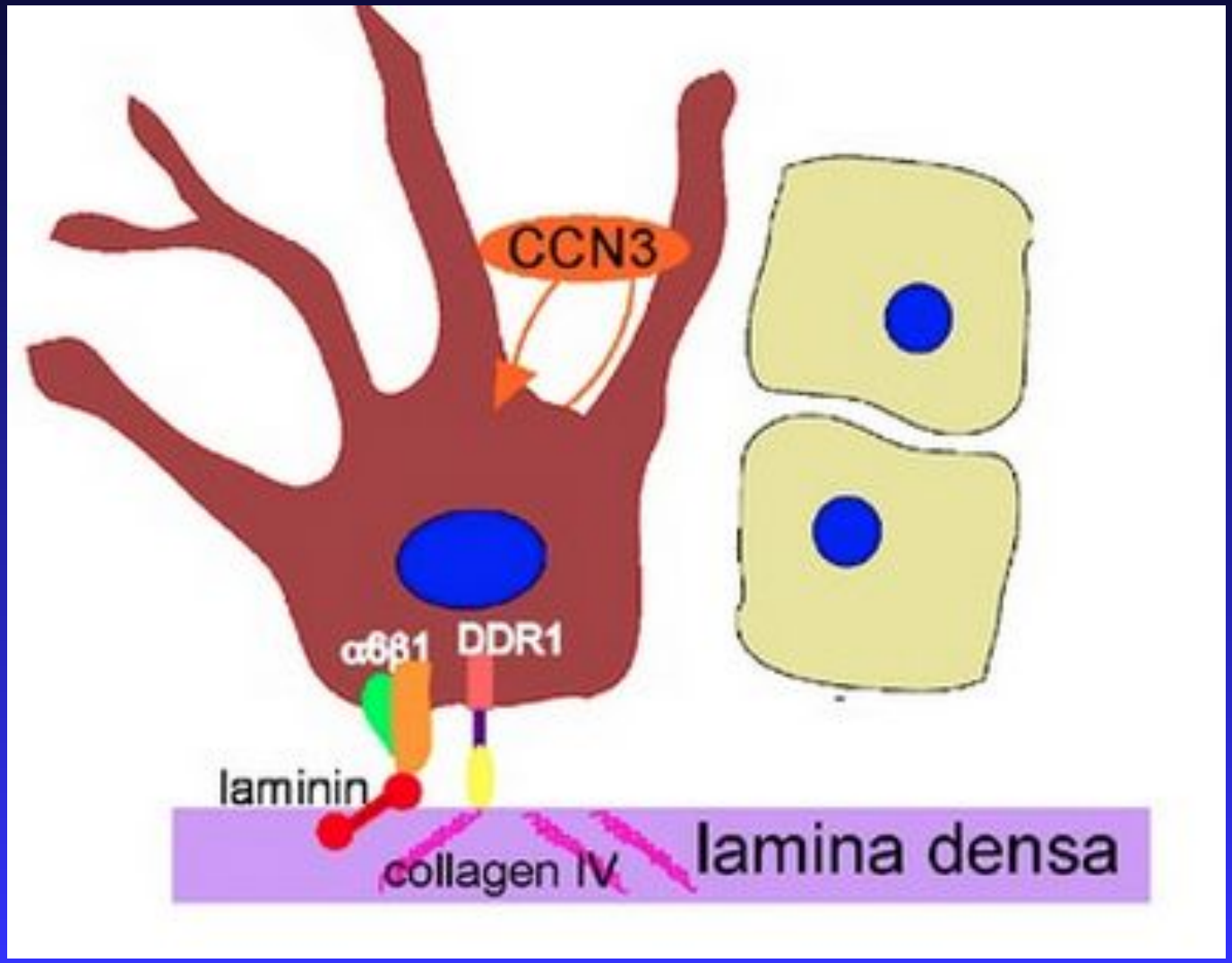
ABSTRACT

Neural crest cells are highly migratory cells that give rise to many derivatives including peripheral ganglia, craniofacial structures and melanocytes. Neural crest cells migrate along defined pathways to their target sites, interacting with each other and their environment as they migrate. Cell adhesion molecules are critical during this process. In this review we discuss the expression and function of cell adhesion molecules during the process of neural crest migration, in particular cadherins, integrins, members of the immunoglobulin superfamily of cell adhesion molecules, and the proteolytic enzymes that cleave these cell adhesion molecules. The expression and function of these cell adhesion molecules and proteases are compared across neural crest emigrating from different axial levels, and across different species of vertebrates.

Neural crest migration

- Cadherins
- Integrins
- Matrix metalloproteases
- IgCAM family

Molecular



CCN3 controls 3D spatial localization of melanocytes in the human skin through DDR1

Mizuho Fukunaga-Kalabis,[1] Gabriela Martinez,[1] Zhao-Jun Liu,[1] Jiri Kalabis,[1] Paul Mrass,[2] Wolfgang Weninger,[2] Sue M. Firth,[3] Nathalie Planque,[4] Bernard Perbal,[4] and Meenhard Herlyn[1]

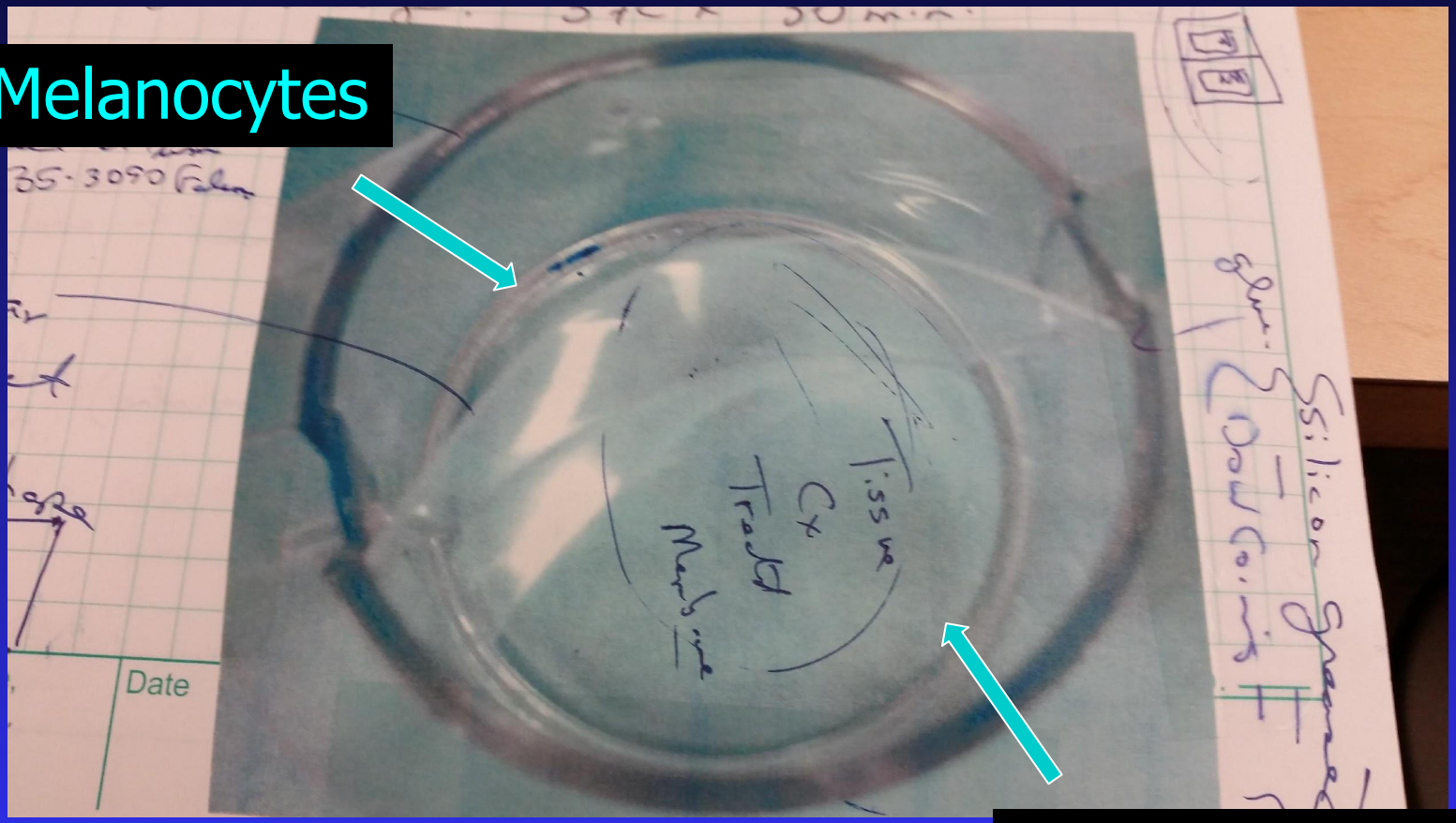
[1]Molecular and Cellular Oncogenesis Program and [2]Immunology Program, The Wistar Institute, Philadelphia, PA 19104
[3]Kolling Institute of Medical Research, University of Sydney, Royal North Shore Hospital, St. Leonards NSW 2065, Australia
[4]Laboratoire d'Oncologie Virale et Moléculaire, Université Paris, 75005 Paris, France

Melanocytes reside within the basal layer of the human epidermis, where they attach to the basement membrane and replicate at a rate proportionate to that of keratinocytes, maintaining a lifelong stable ratio. In this study, we report that coculturing melanocytes with keratinocytes up-regulated CCN3, a matricellular protein that we subsequently found to be critical for the spatial localization of melanocytes to the basement membrane. CCN3 knockdown cells were dissociated either upward to the suprabasal layers of the epidermis or downward into the dermis. The overexpression of CCN3 increased adhesion to collagen type IV, the major component of the basement membrane. As the receptor responsible for CCN3-mediated melanocyte localization, we identified discoidin domain receptor 1 (DDR1), a receptor tyrosine kinase that acts as a collagen IV adhesion receptor. DDR1 knockdown decreased melanocyte adhesion to collagen IV and shifted melanocyte localization in a manner similar to CCN3 knockdown. These results demonstrate an intricate and necessary communication between keratinocytes and melanocytes in maintaining normal epidermal homeostasis.

Developing a model for in-vitro migration of melanocytes.

- Co-culture of melanocytes and keratinocytes.
  - Proliferation and movement.
  - Molecular mechanisms
  - Agents that induce or suppress movement

Co-culture in-vitro model



H. Decean · M. Perde-Schrepler · C. Tatomir · E. Fischer-Fodor · I. Brie · P. Virag

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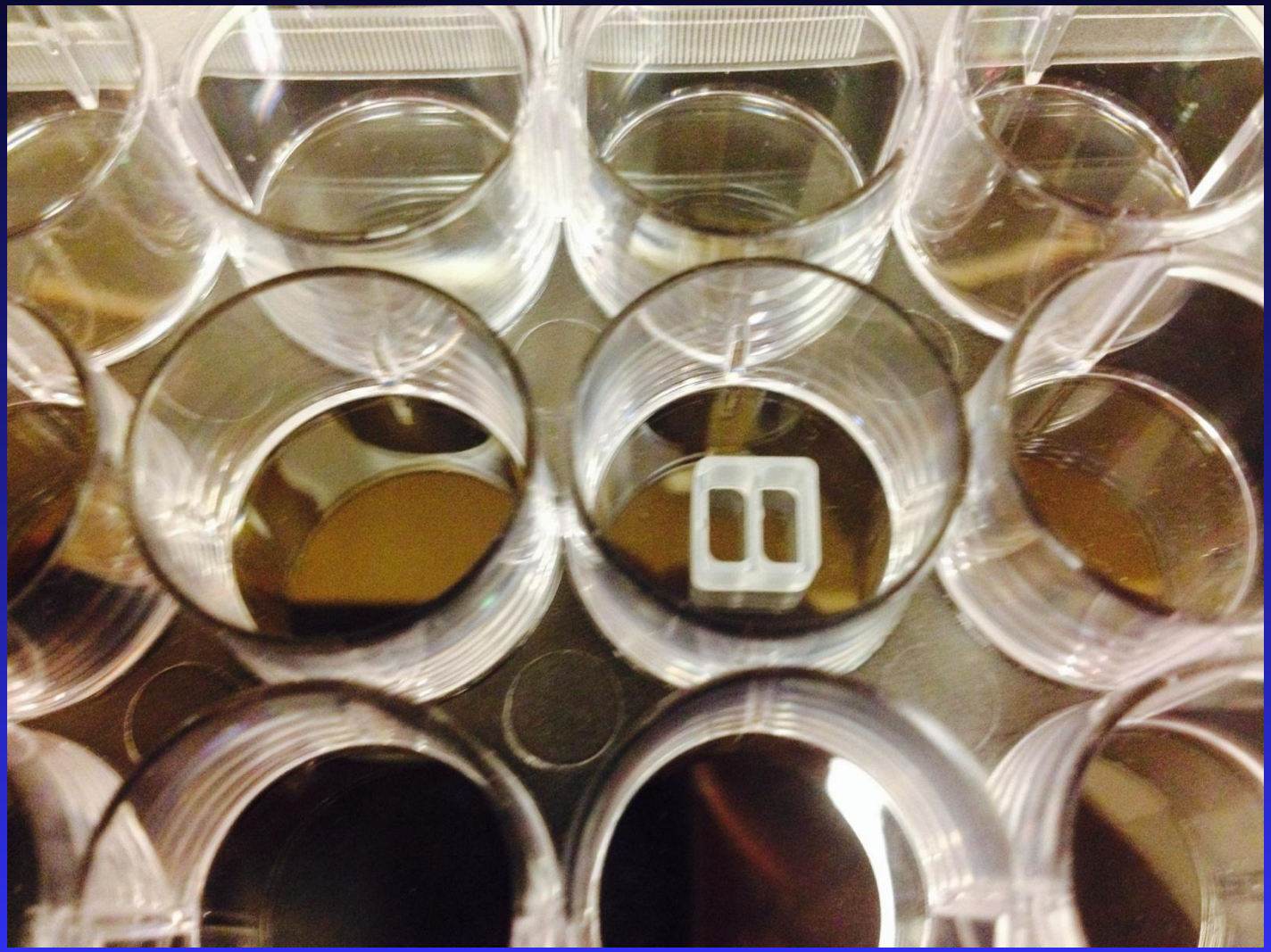
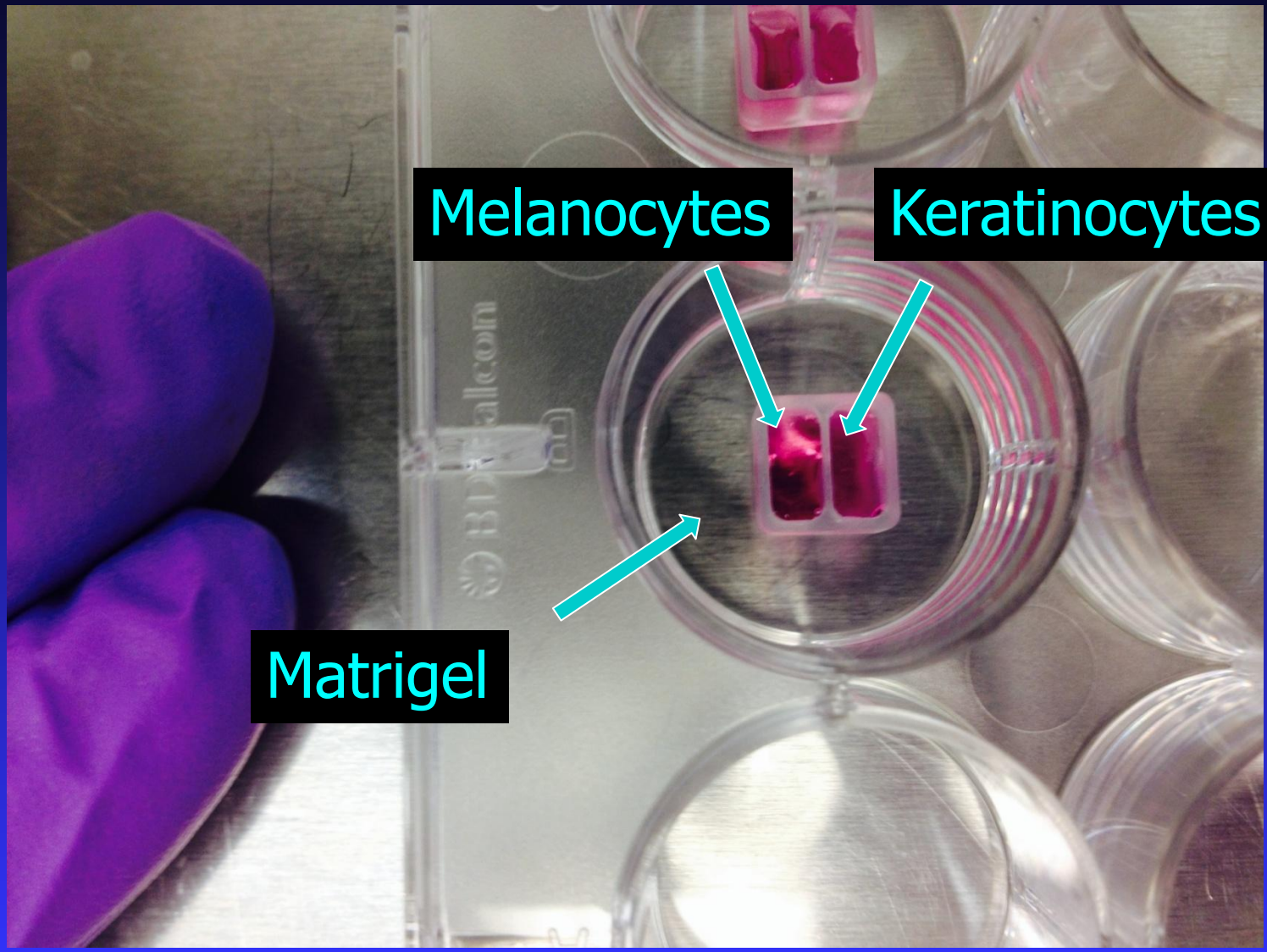
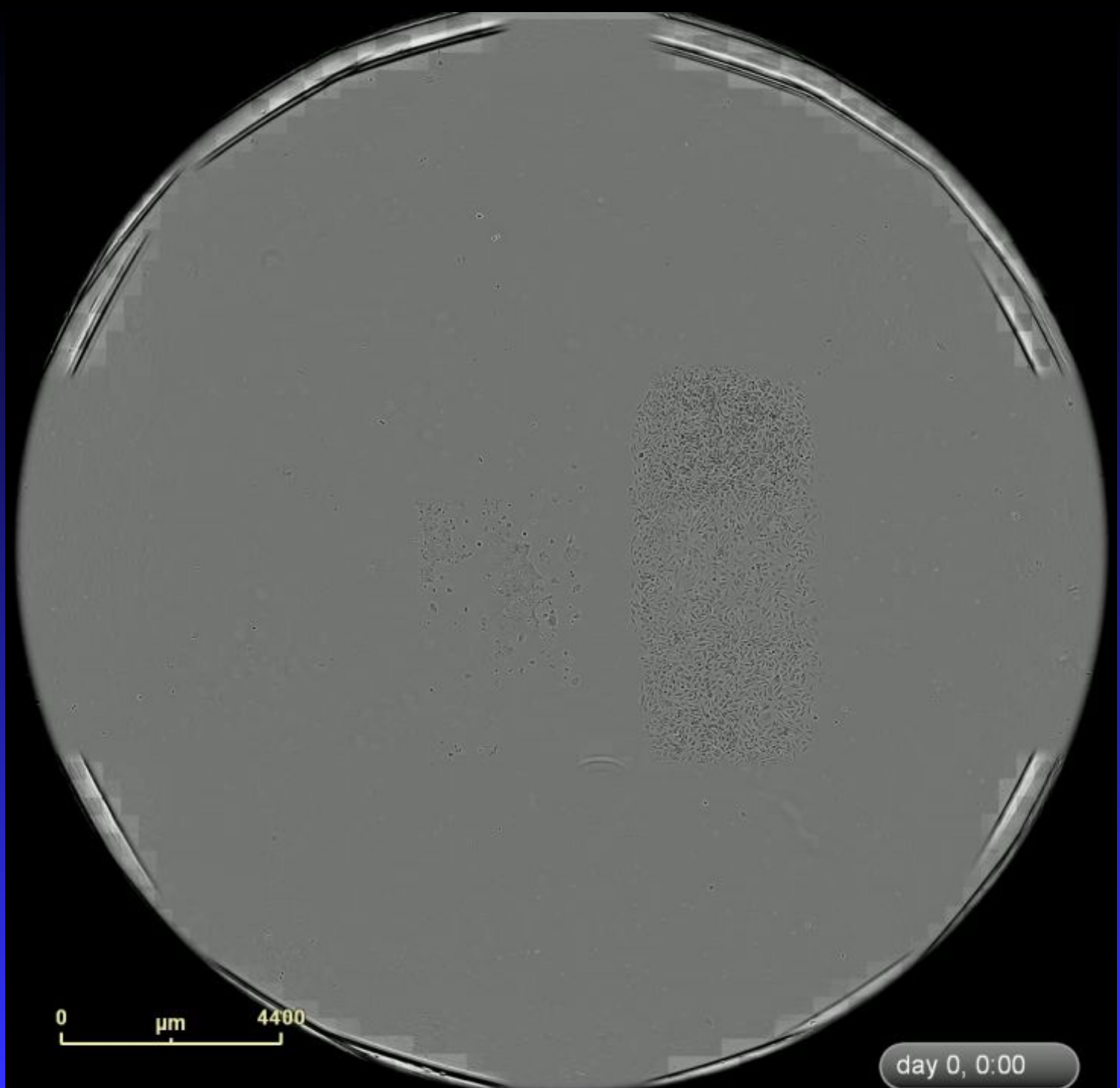
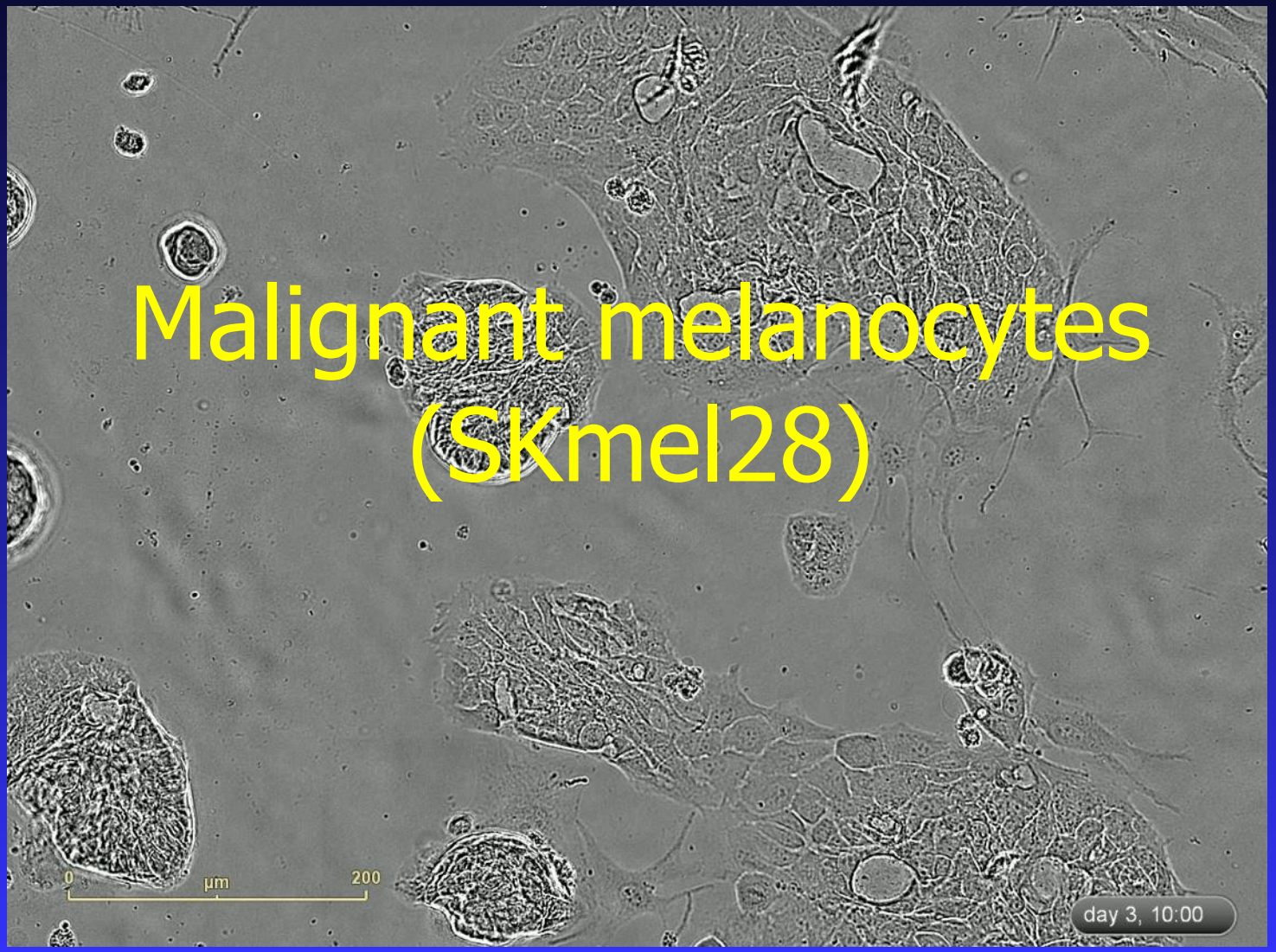
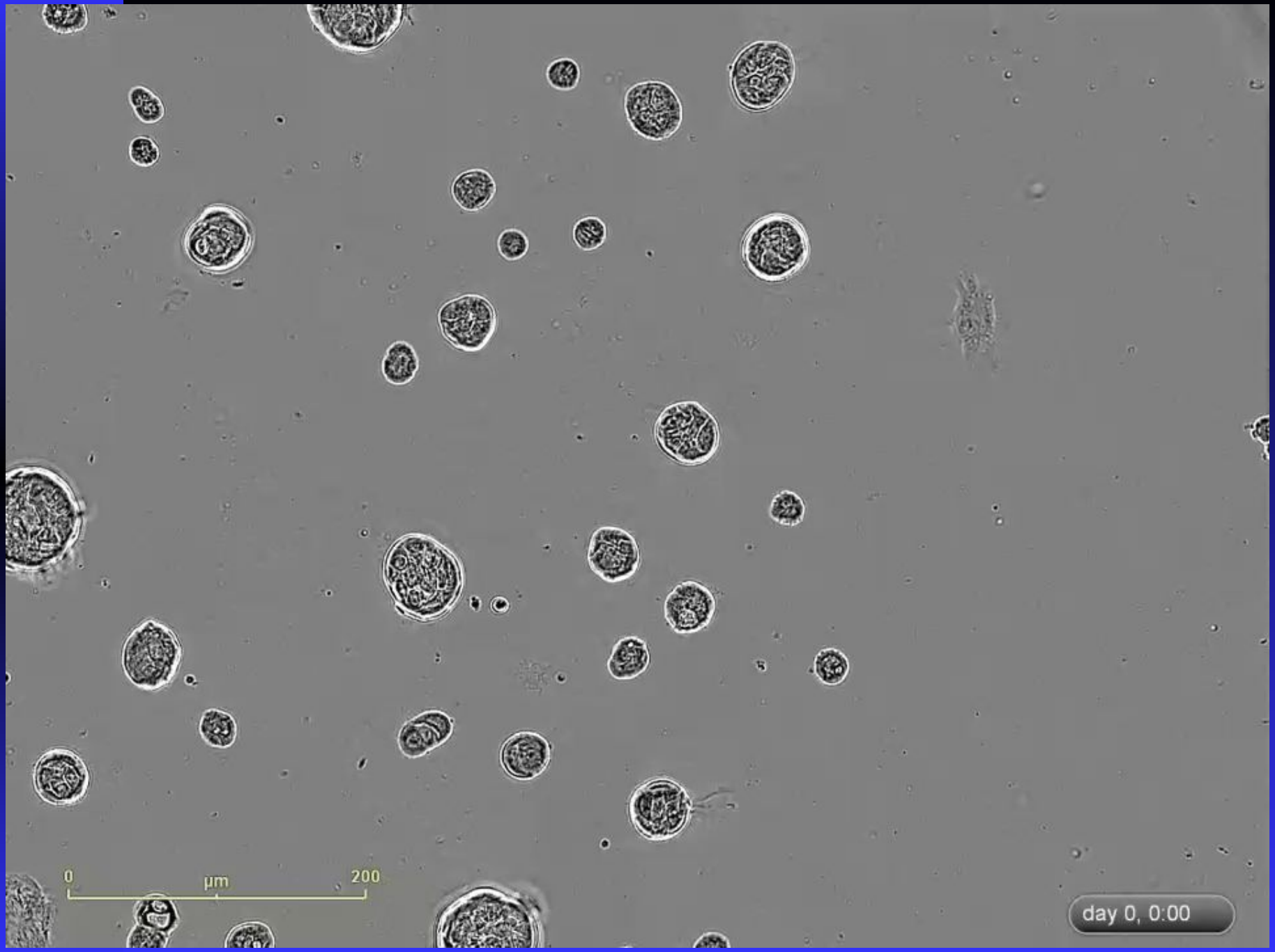
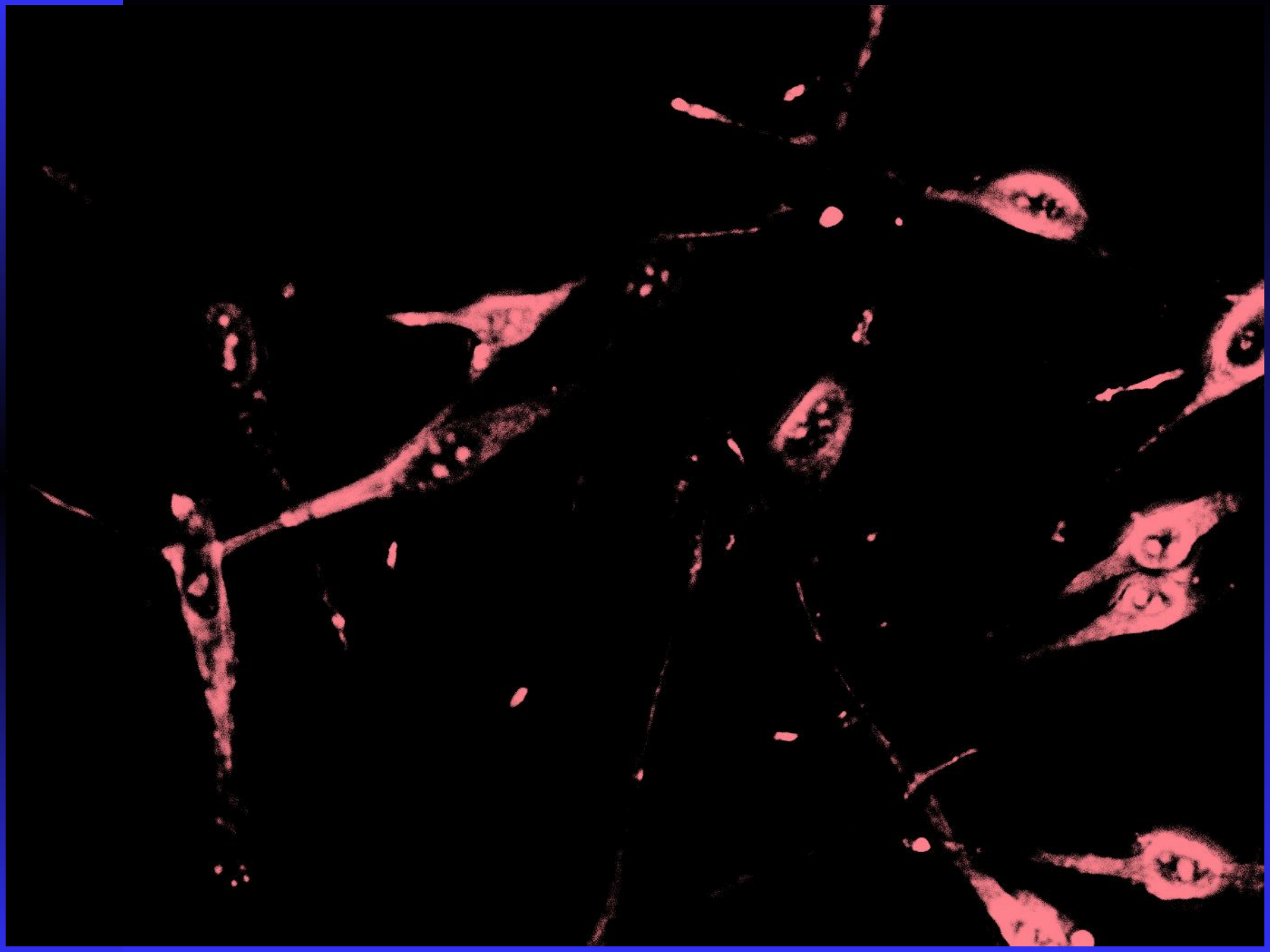



Melanocytes
Keratinocytes
Matrigel

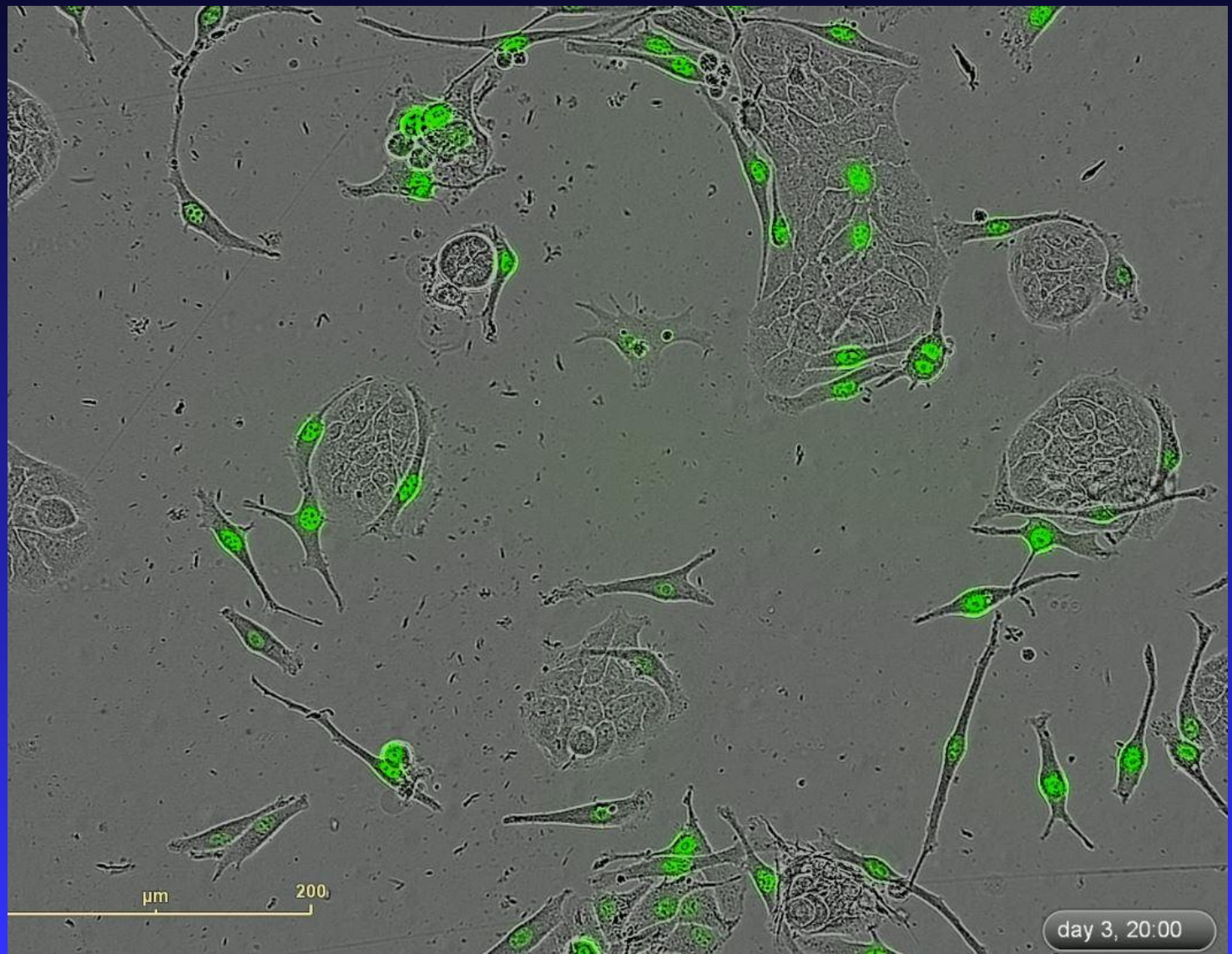

0
µm
4400
day 0, 0:00


Malignant melanocytes
(SKmel28)
0
µm
200
day 3, 10:00

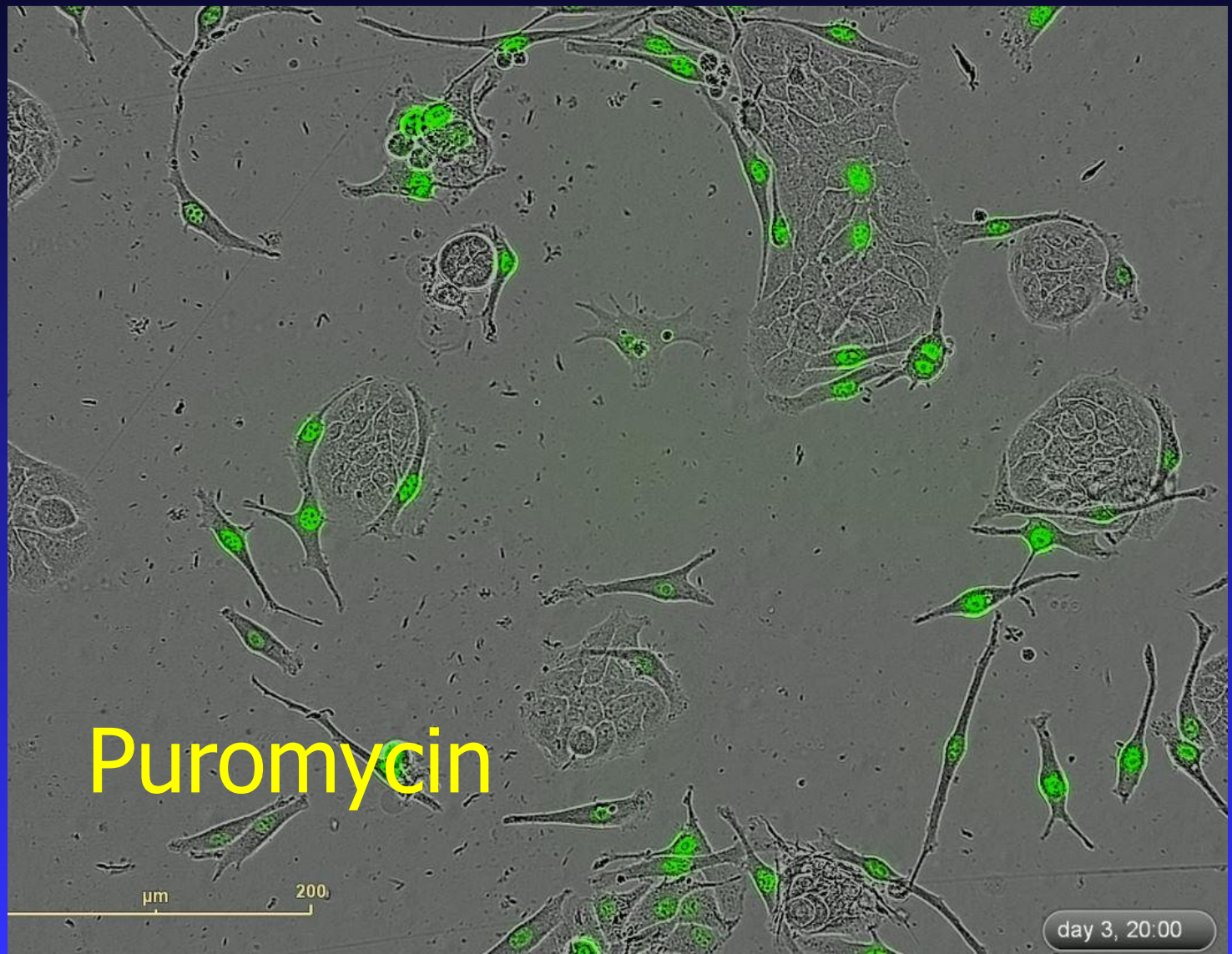

0
µm
200
day 0, 0:00

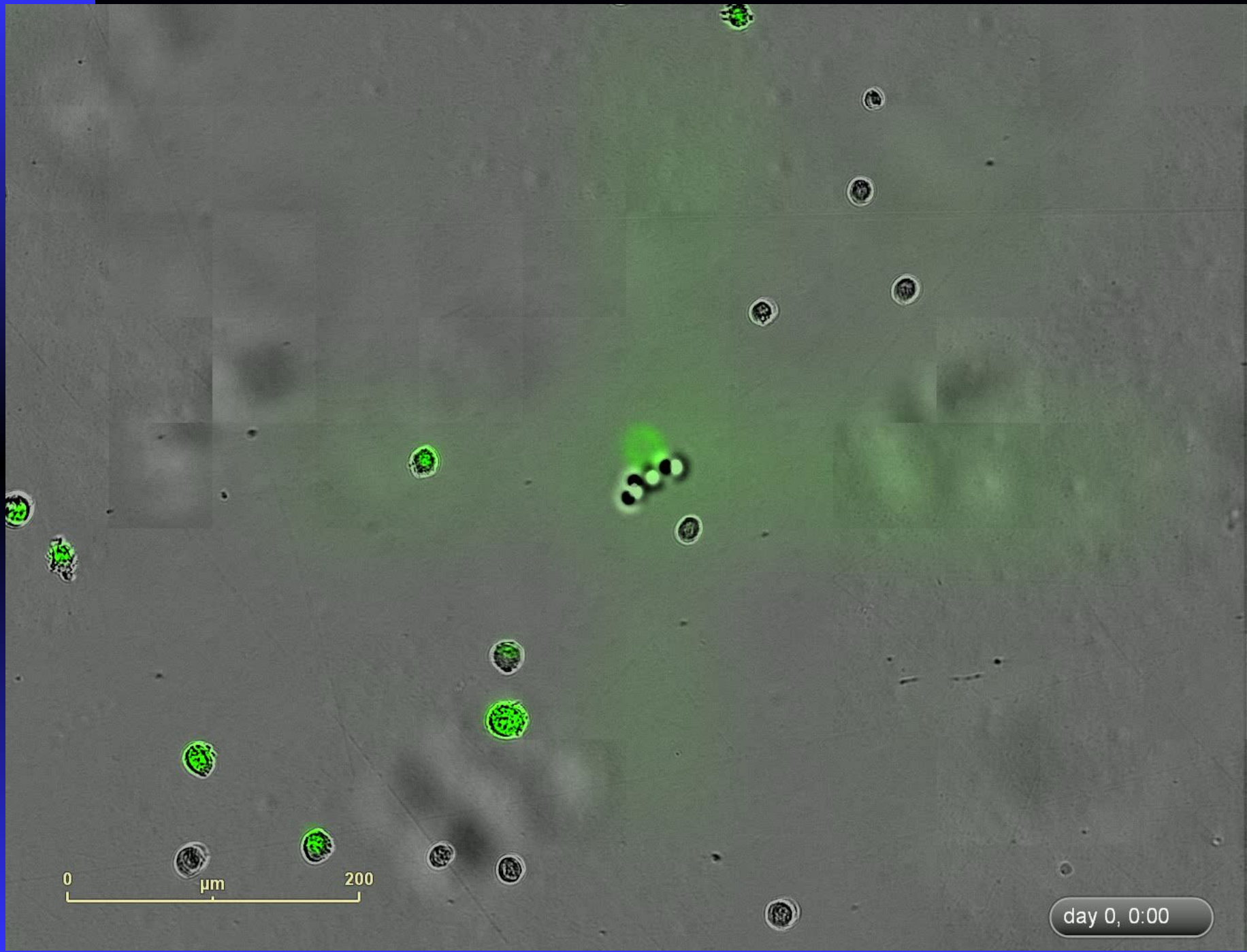


Malignant—SKmel28GFP

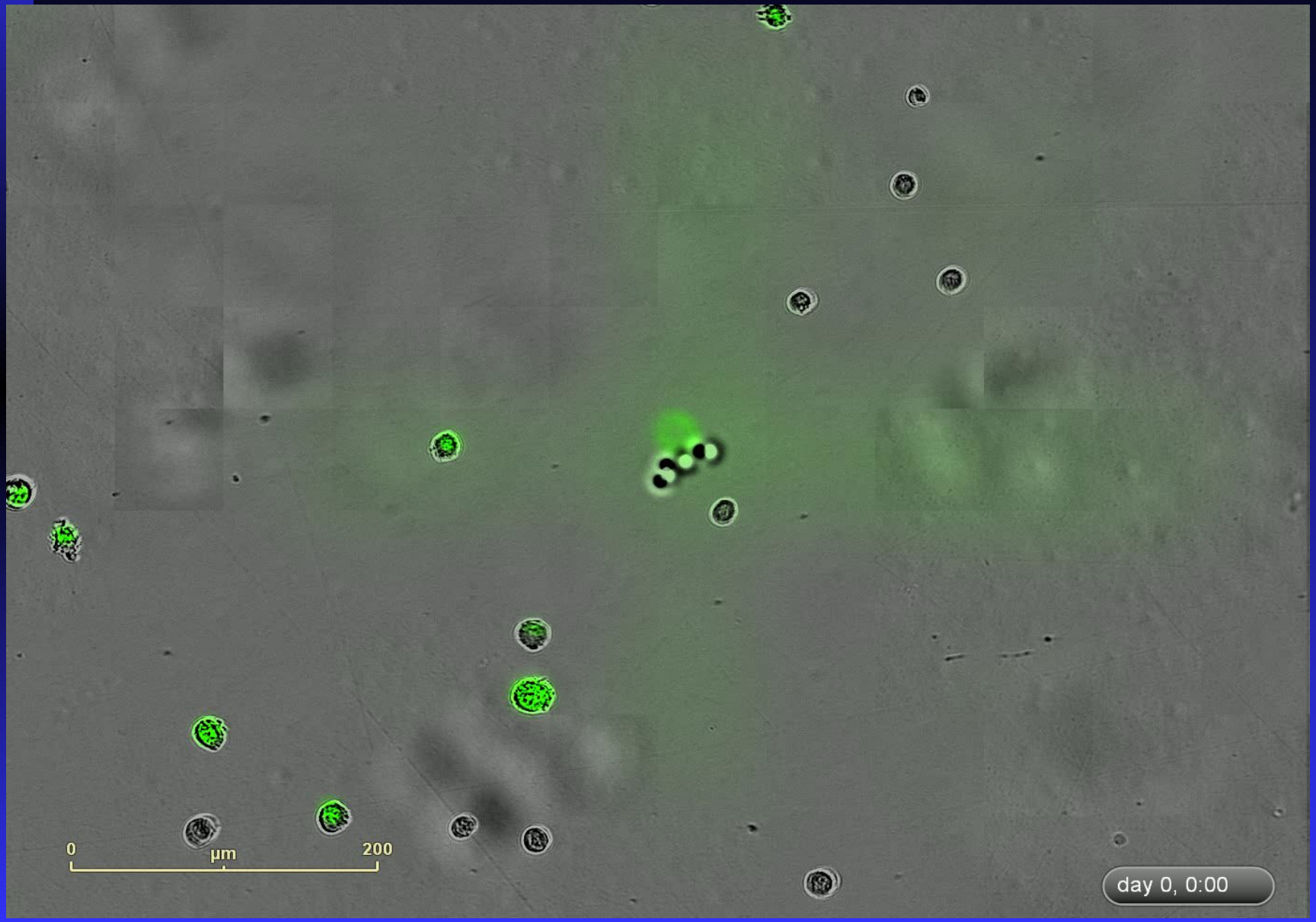


Malignant—SKmel28GFP

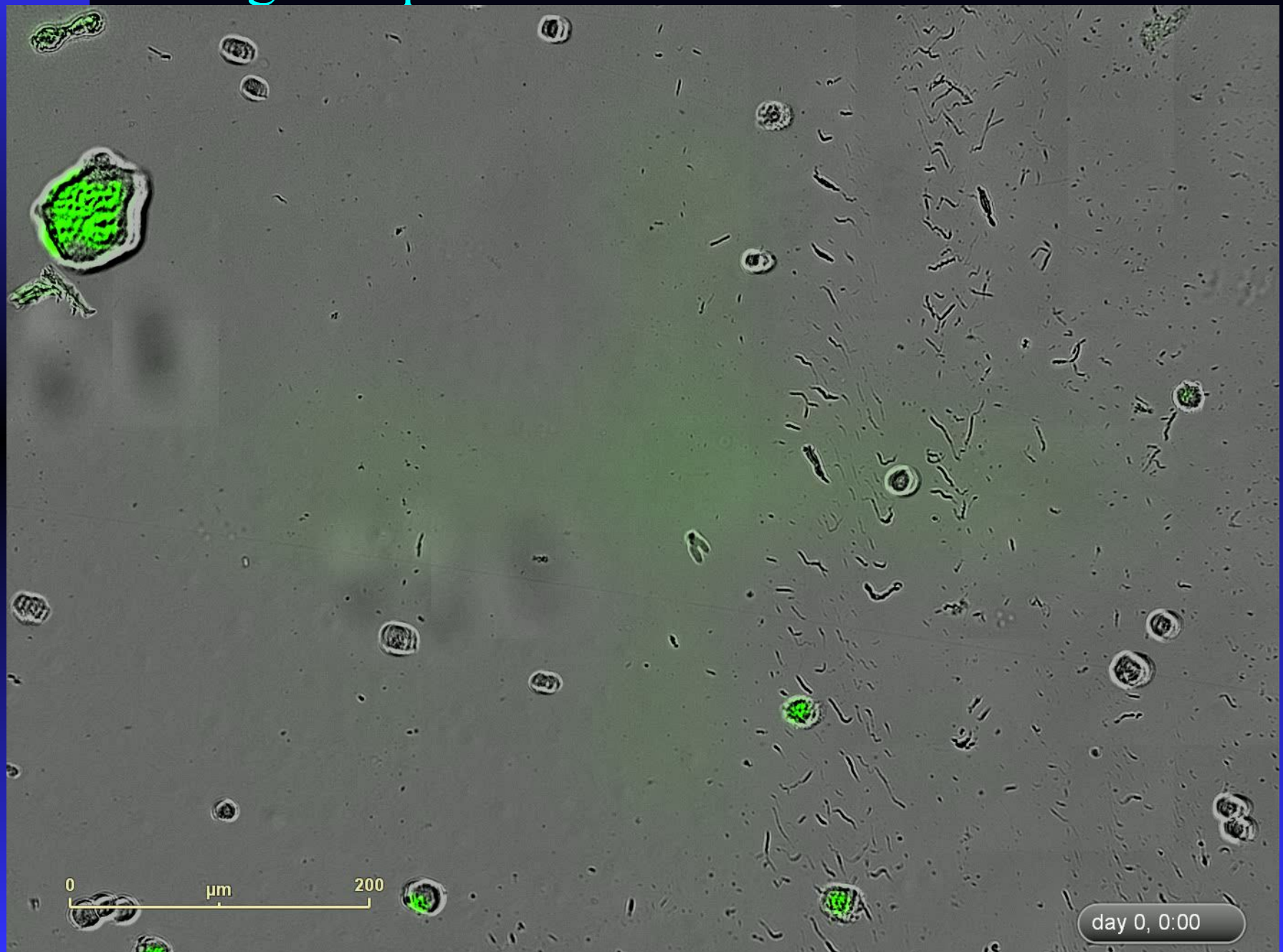


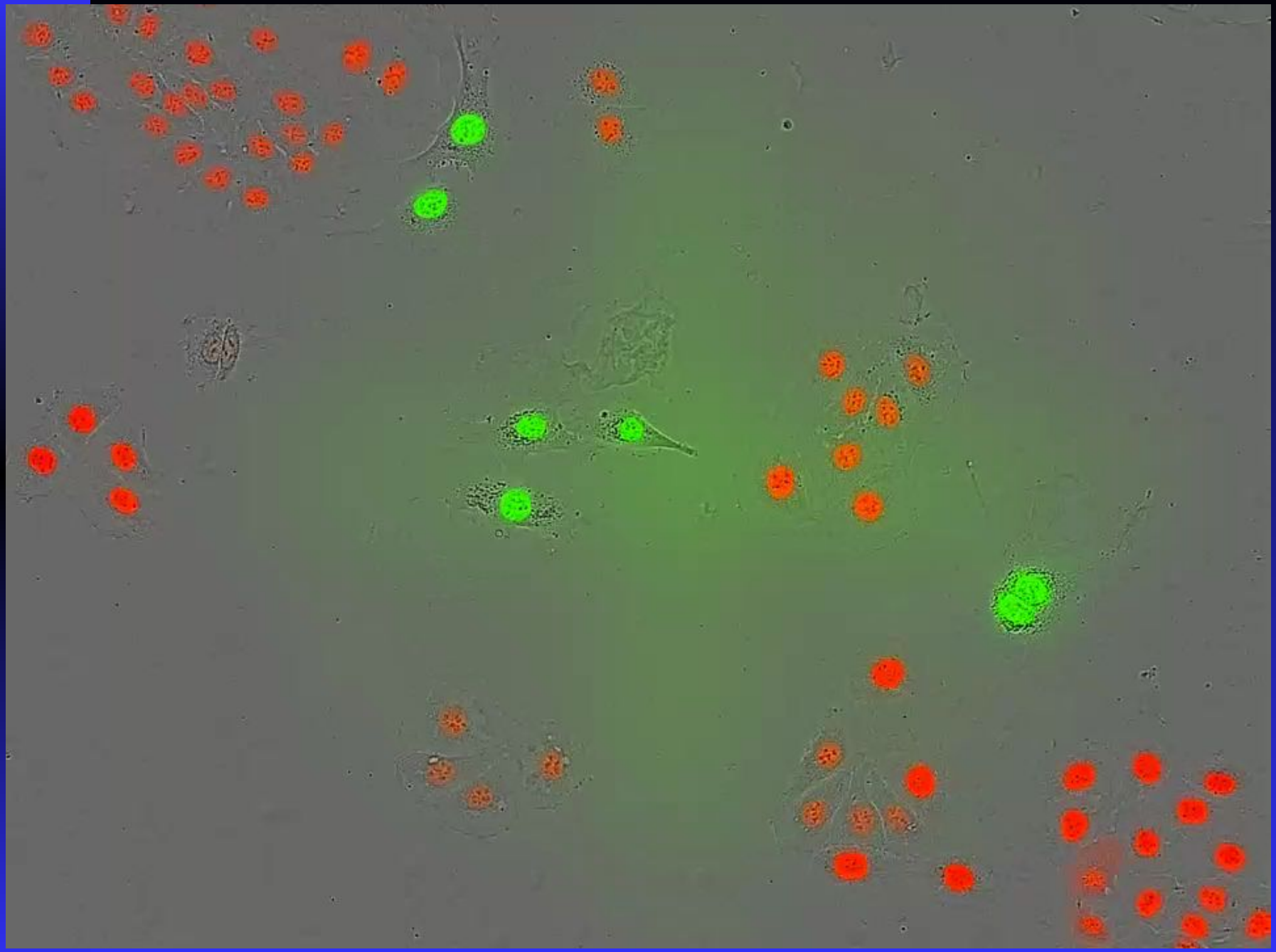

0
µm
200
day 0, 0:00

Minimal interaction of the cells.



Matrigel required



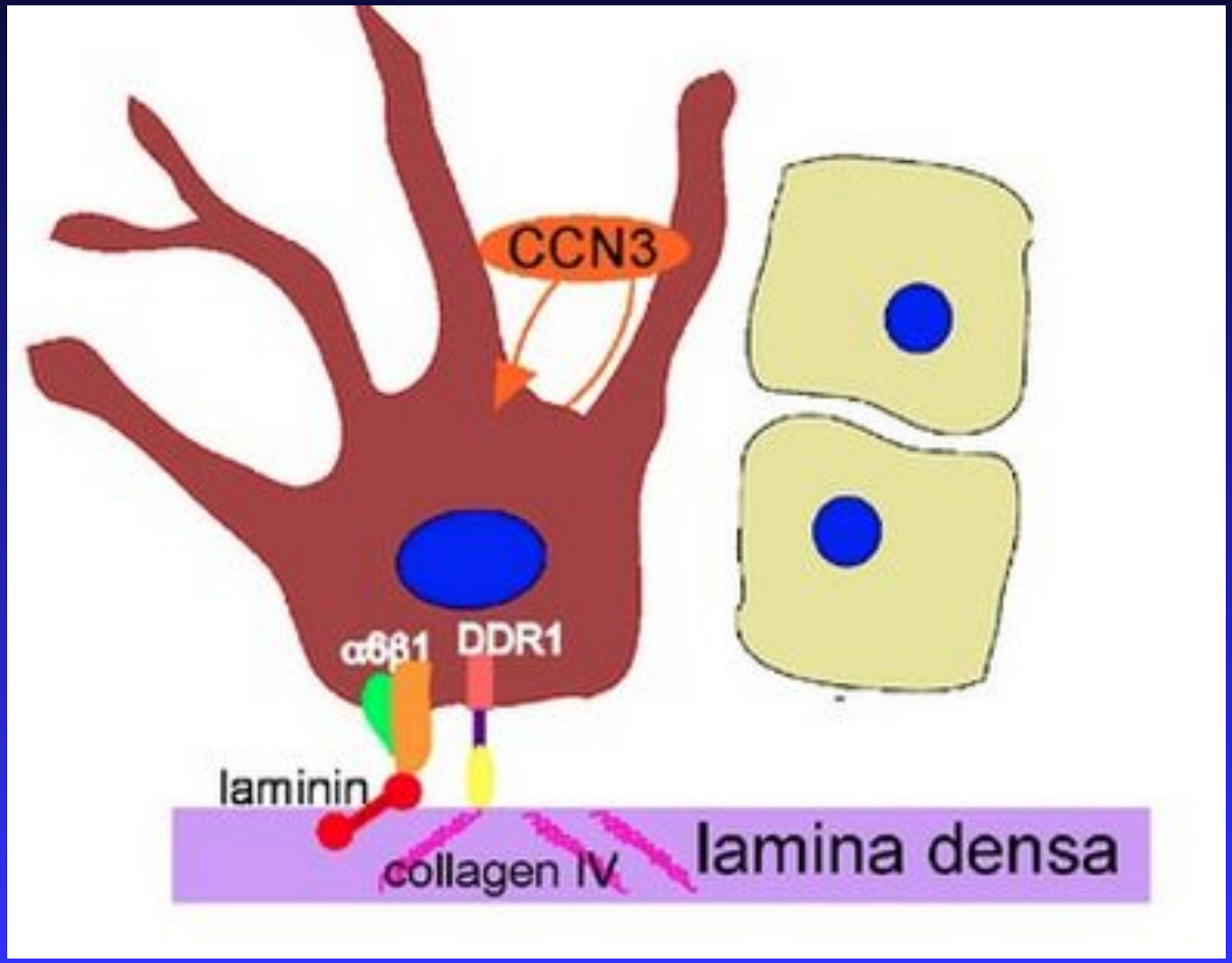


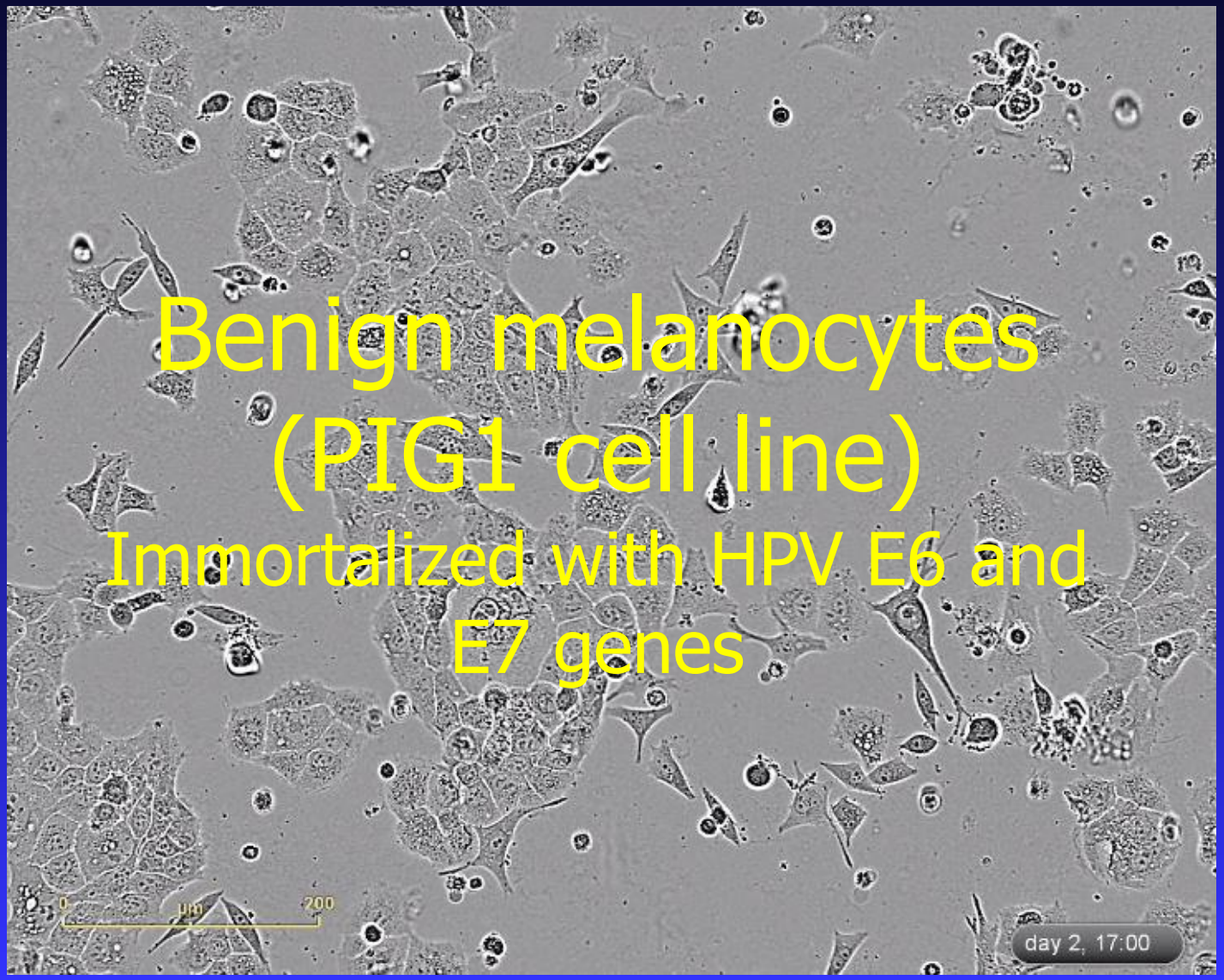
Matrigel—In process of testing components

- Laminin
- Beta-NGF
- Nidogen-1/Entactin
- Heparan sulfate

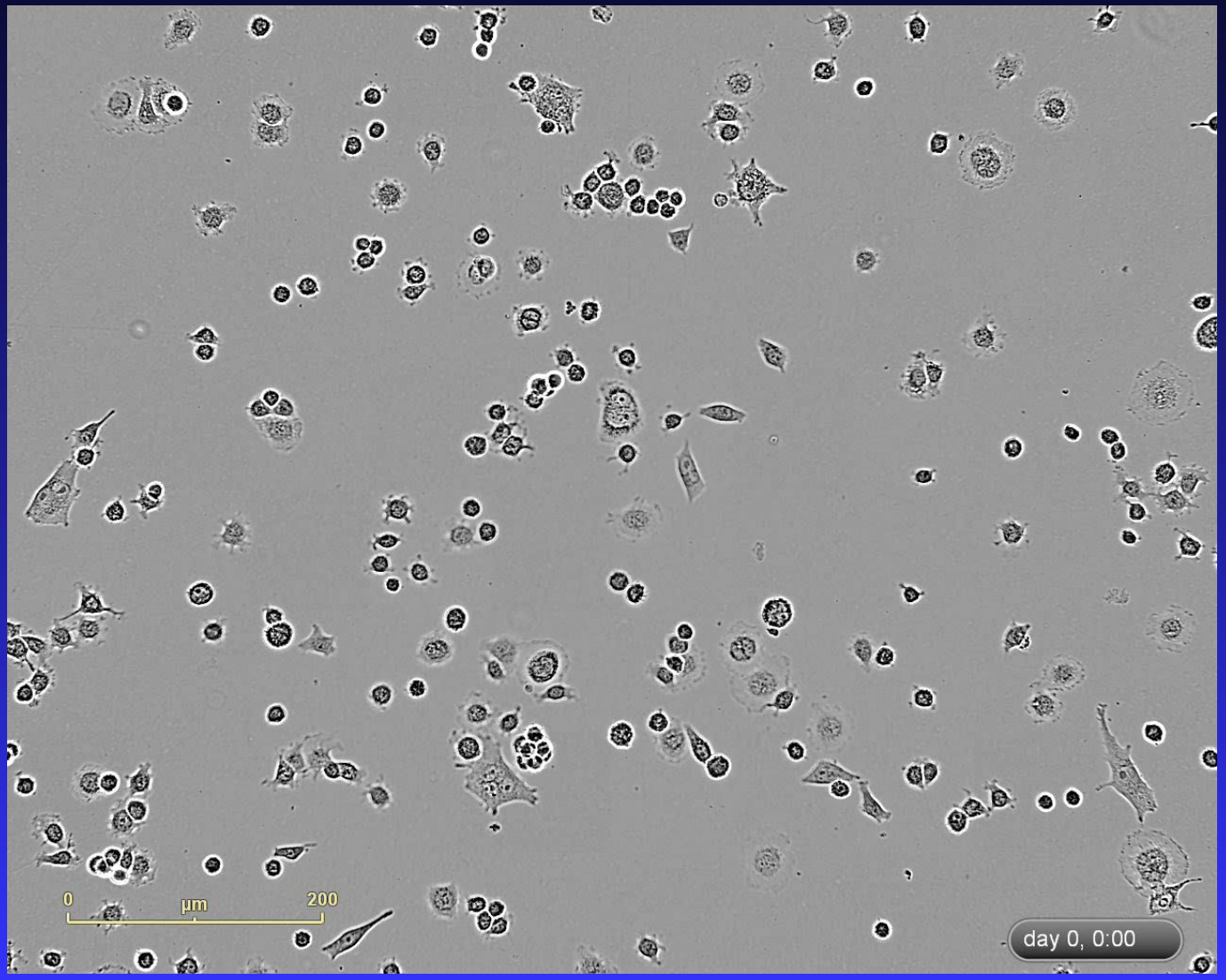
- Collagen IV—No interaction

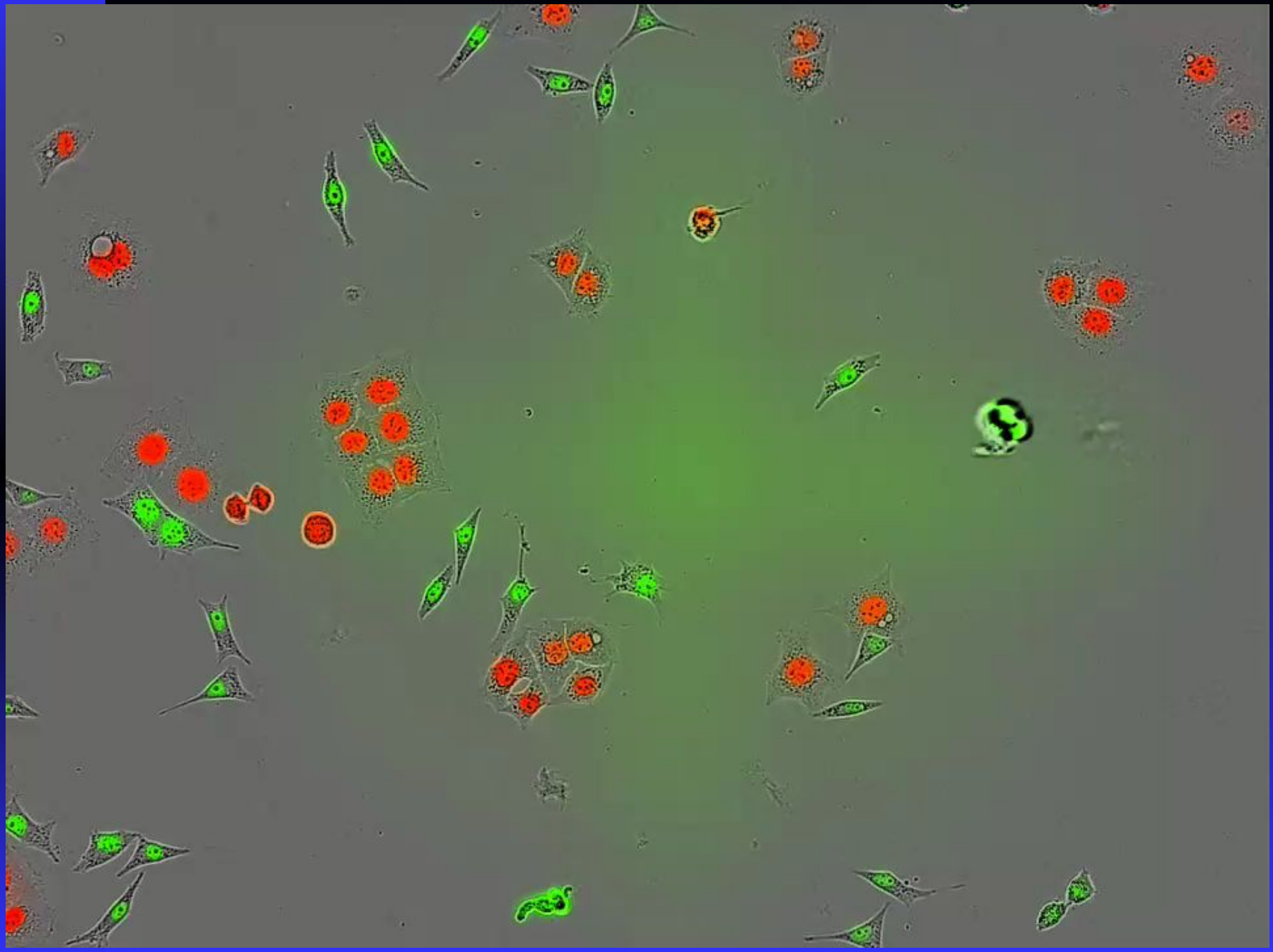
Molecular



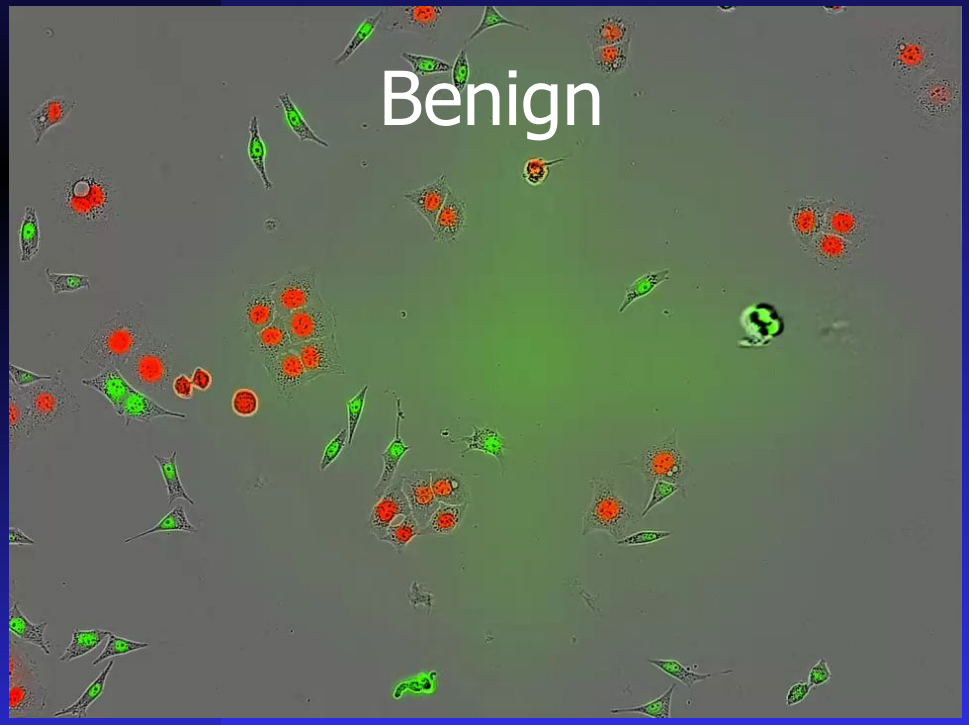

Benign melanocytes
(PIG1 cell line)
Immortalized with HPV E6 and
E7 genes
0
200
day 2, 17:00

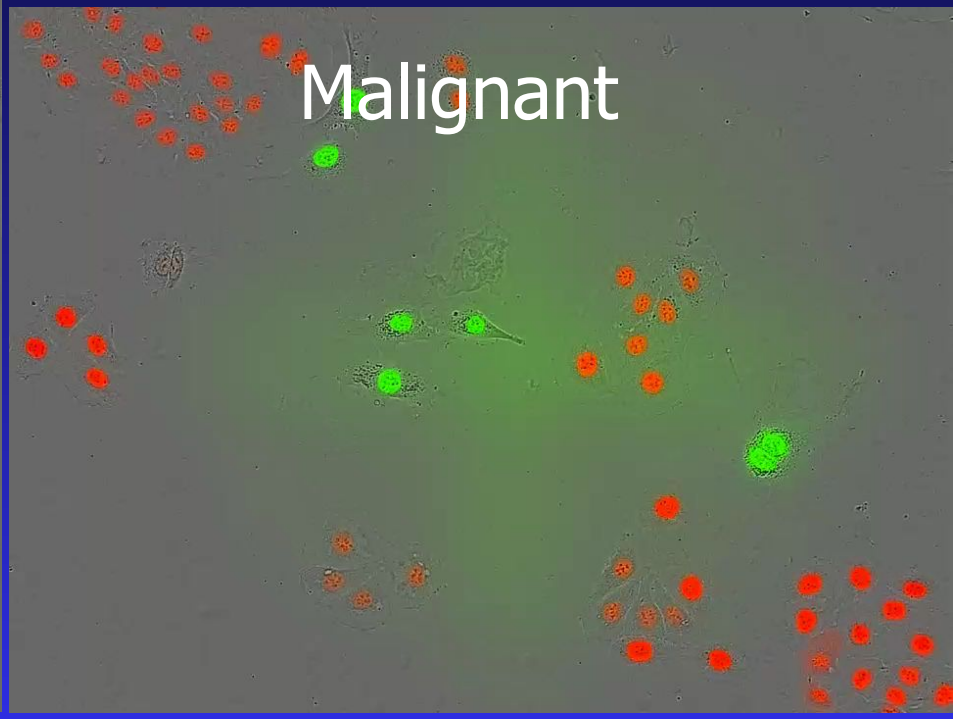
PIG1





Malignant melanocytes move more than benign melanocytes

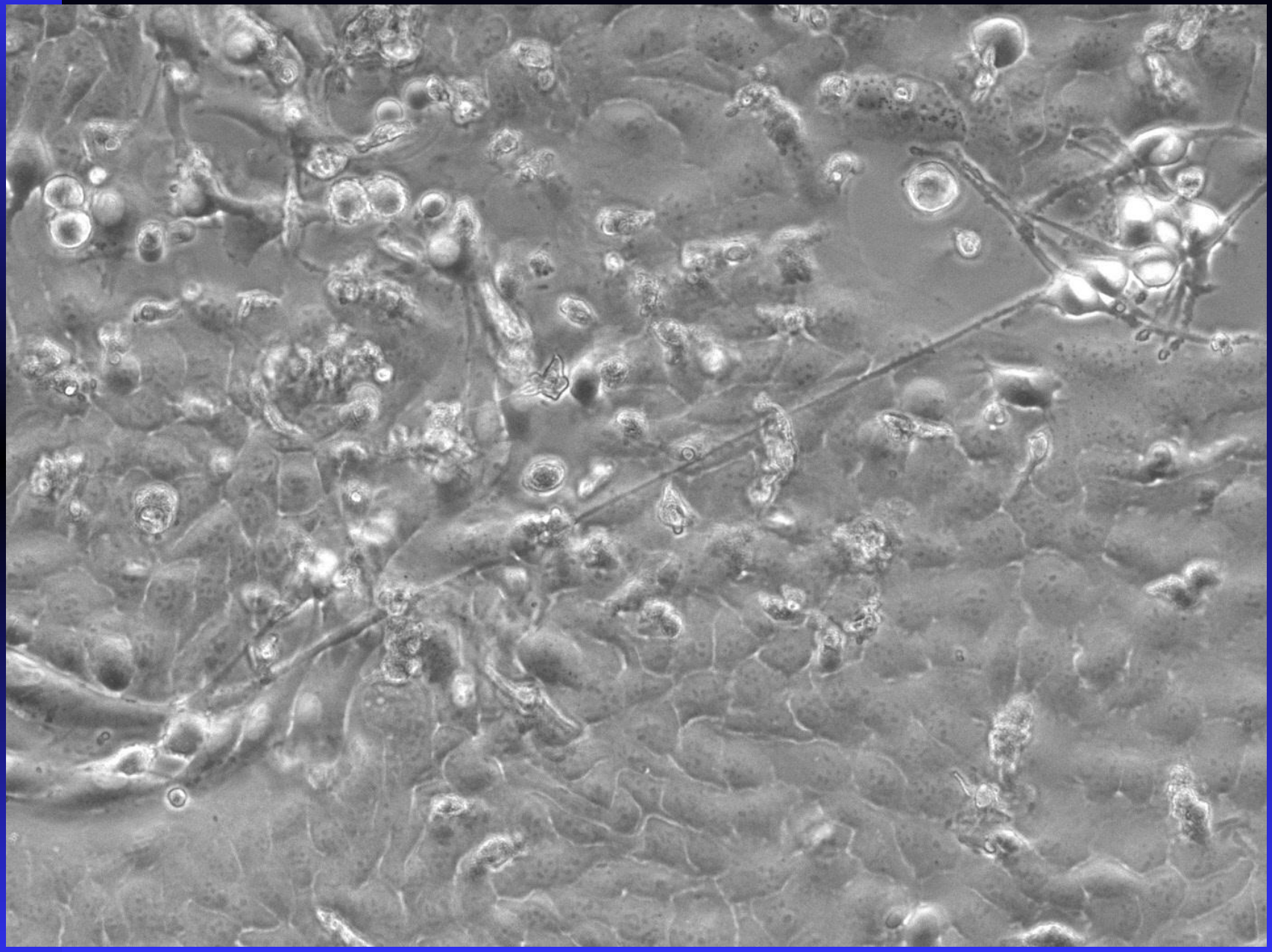




Co-culture Media

- Keratinocytes (HaCaT)—DMEM or EMEM with 10%FBS
- Melanocytes (Malignant)—EMEM10%FBS
- Melanocytes (Benign)—HAM F10, 7.5%FBS, Isobtyl-methylxanthin, PCN, sodium orthovanadate, cholera toxin, PMA (PIG1 media)

3:1 EMEM10%FBS:PIG1



Plans for the Co-culture Model

- Define movement and interaction
  - Transfer or melanosomes (endocytosis?)
- Testing of agents
  - Inhibit
  - Stimulate
- Understanding cell-matrix (environment)

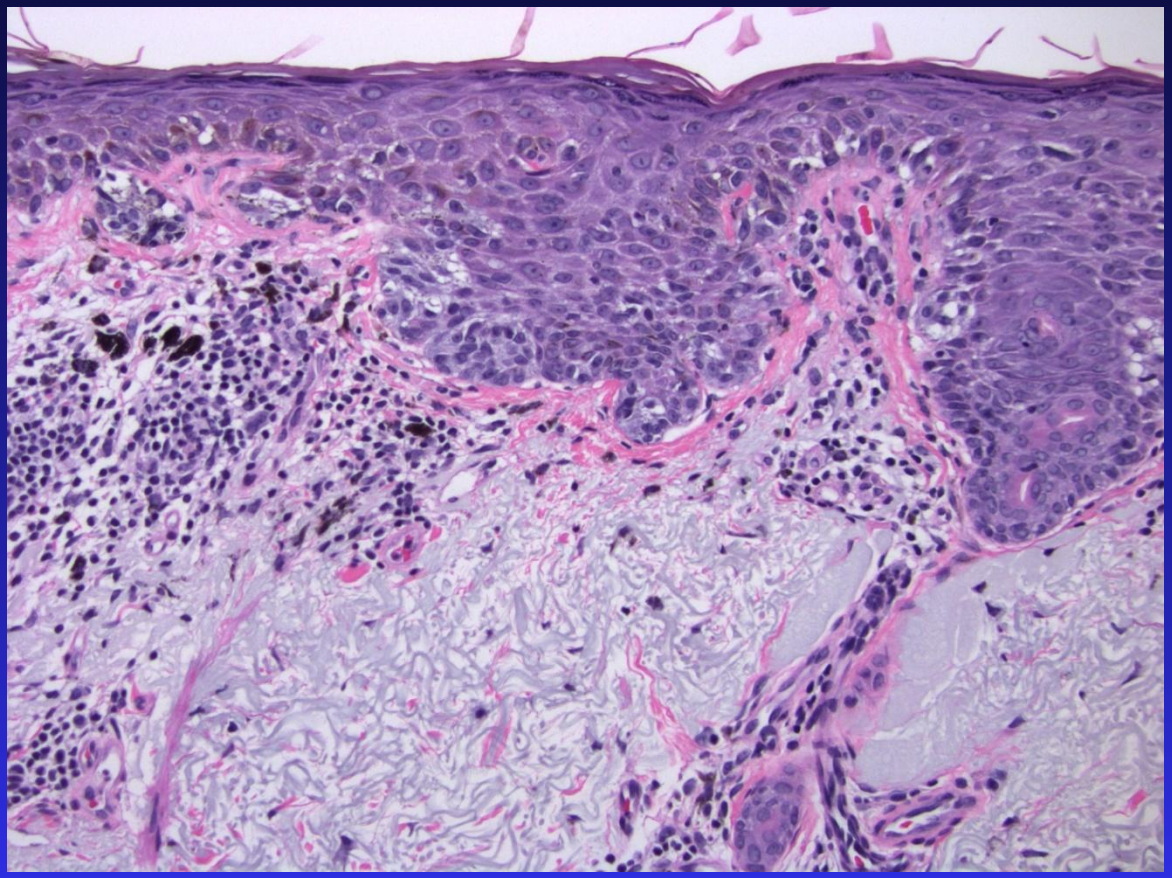
Stimulation of Pigmentation

- Piperine
  - Black pepper
  - Unknown mechanism
    - Inhibition of CYP3A4, P-glycoprotein y “enzymes”
    - Important in the metabolism and transportation of molecules.

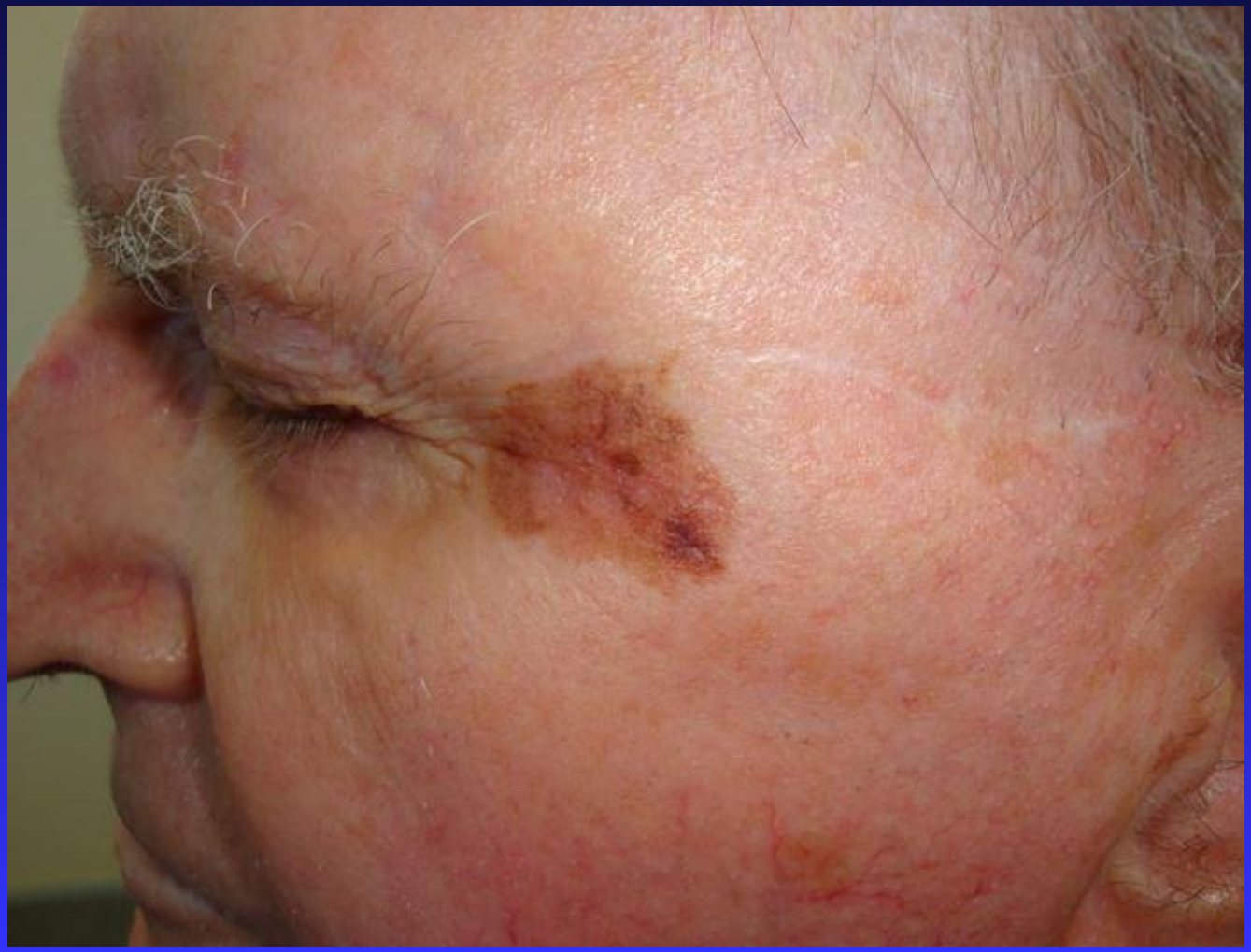
Types of melanoma

- Lentigo maligna—chronic sun—face/neck
- Superficial spreading--sunburns
- Nodular—sunburns
- Acral lentiginous—hand/foot—dark skin

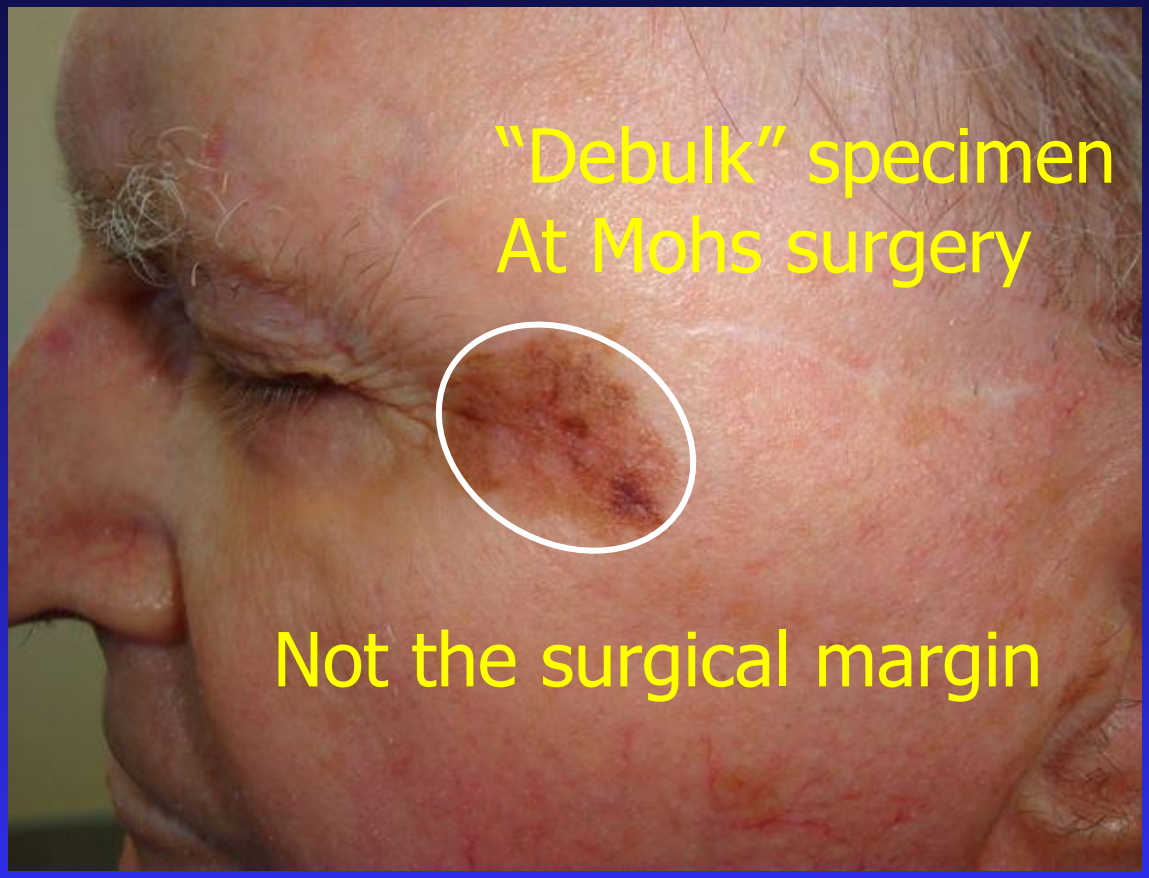
Lentigo Maligna (Melanoma in-situ)



Lentigo Maligna (Melanoma in-situ)
A malignant but benign tumor



Lentigo Maligna (Melanoma in-situ)

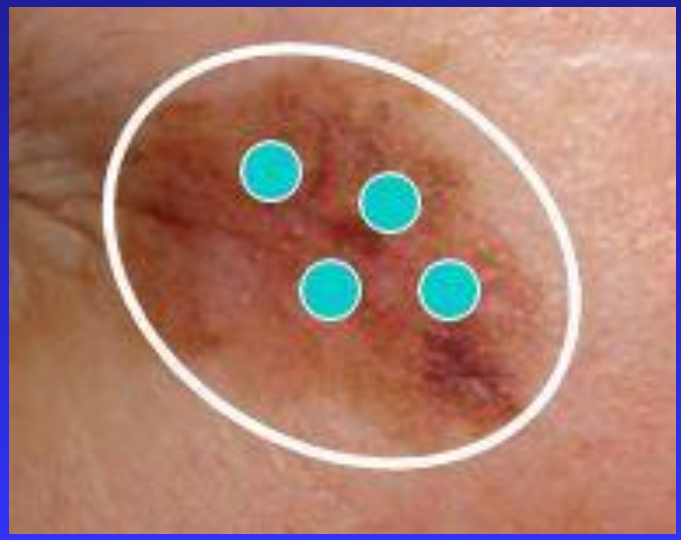


Lentigo Maligna (Melanoma in-situ)

(Added to existing squamous cell carcinoma IRB of Molly Kulesz-Martin)

Fresh tissue--MIS-LM—2 cases and normal control tissue

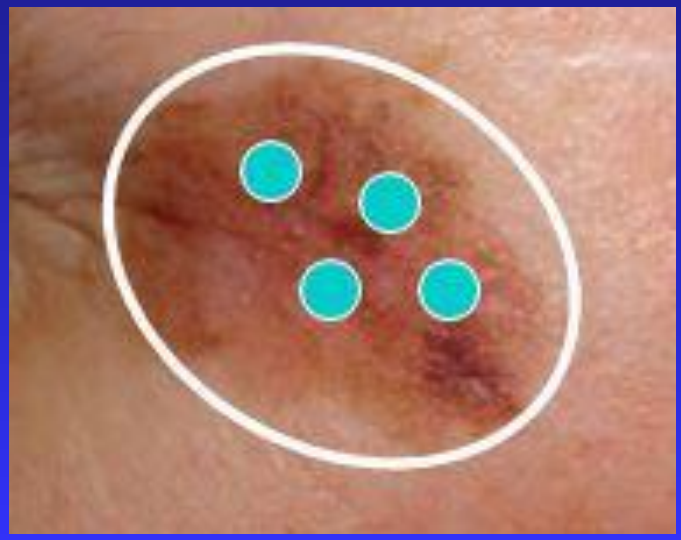
- Electron microscopy
  - Morphology
  - Immunomorphological
- Establish cell lines
- Frozen (OCT) specimen
- Pathology specimen

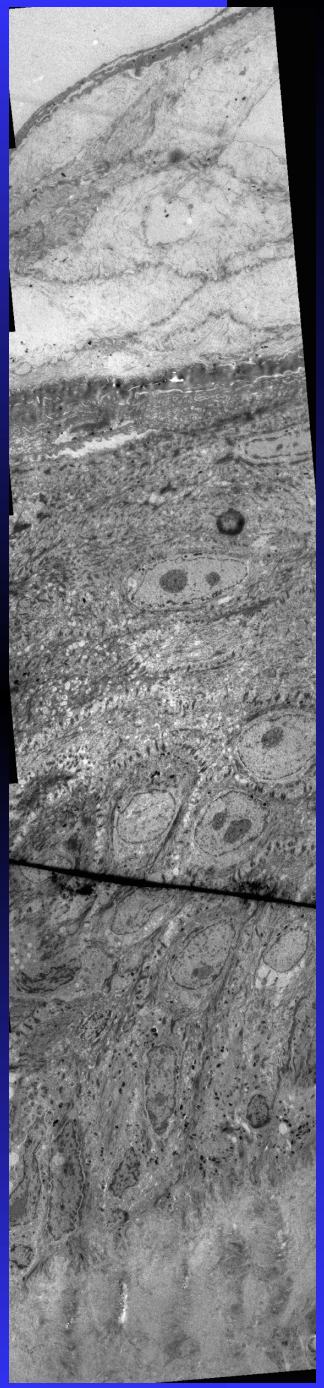


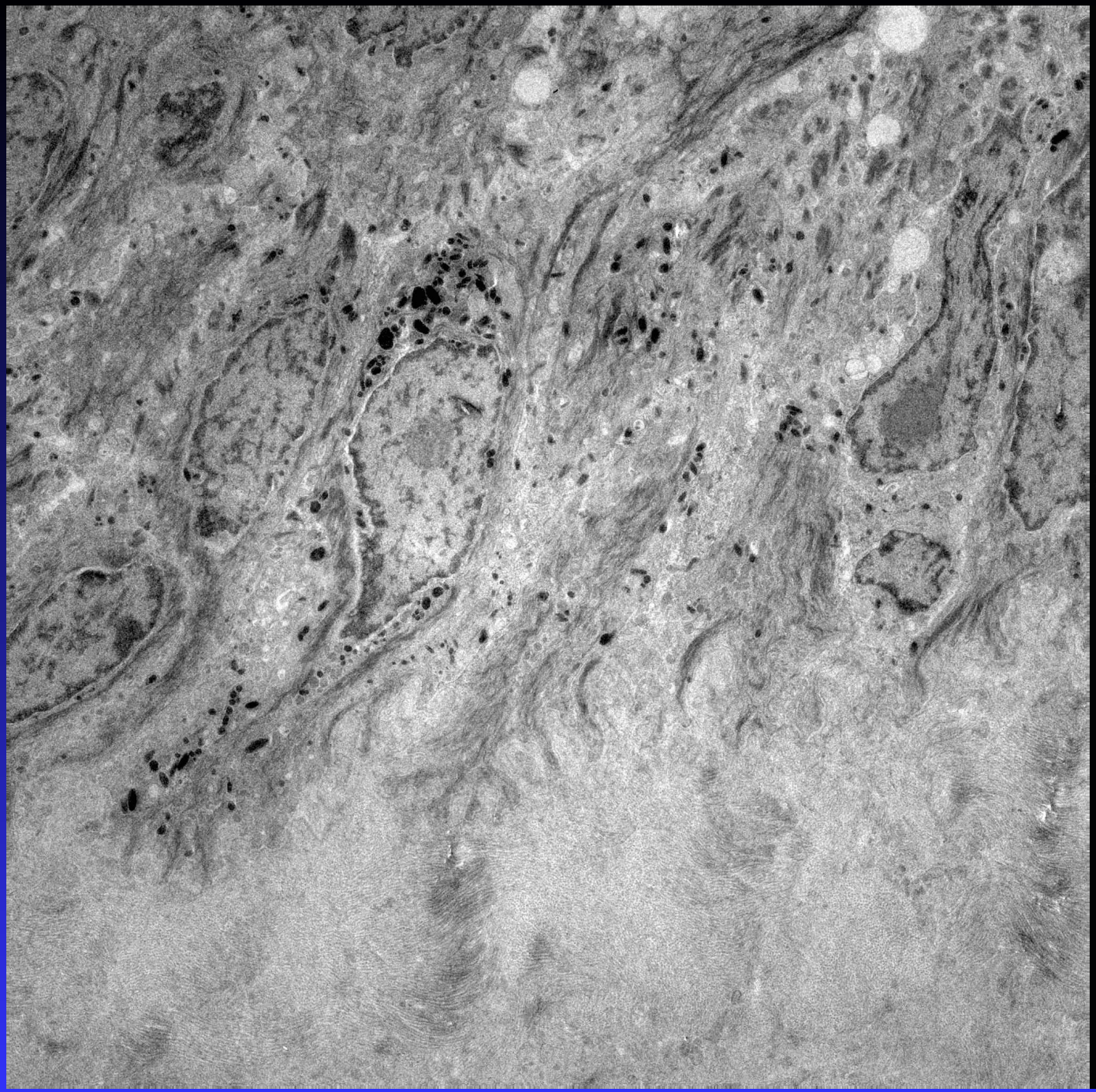
Fresh tissue--MIS-LM—2 cases and normal control tissue

- Electron microscopy
  - Morphology
  - Immunomorphological
- Establish cell lines

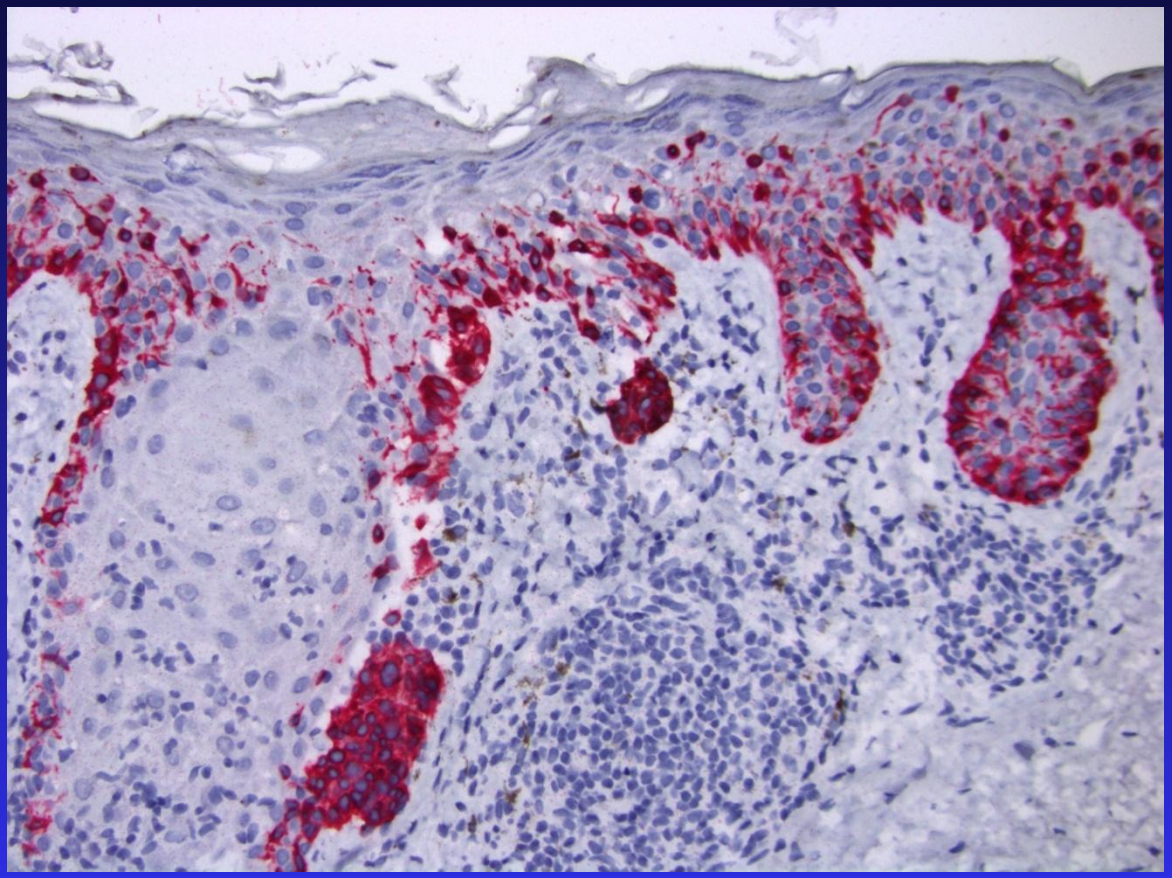
The only source of fresh tissue from melanoma






5 um

Laser microdissection for DNA/RNA analysis—3 cases then expand



Laser Microdissection

What are we going to find?

- A single oncogenic mutation driving proliferation and movement?
  - CCN1/DDR3?
  - Upstream regulatory genes?
  - The “Usual players” in melanoma?

Utility

- New treatments (topical)
  - Mechanism or to stop (or stimulate) movement and proliferation of the melanocytes.
- Development of agents that promote movement for repigmentation.
- Development of tests that can distinguish good MIS from bad MIS.

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