Niels Grabe

TOWARDS QUANTITATIVE SYSTEMS BIOLOGICAL TISSUE MODELS BY USING WHOLE SLIDE IMAGING

TOWARDS QUANTITATIVE SYSTEMS BIOLOGICAL TISSUE MODELS BY USING WHOLE SLIDE IMAGING

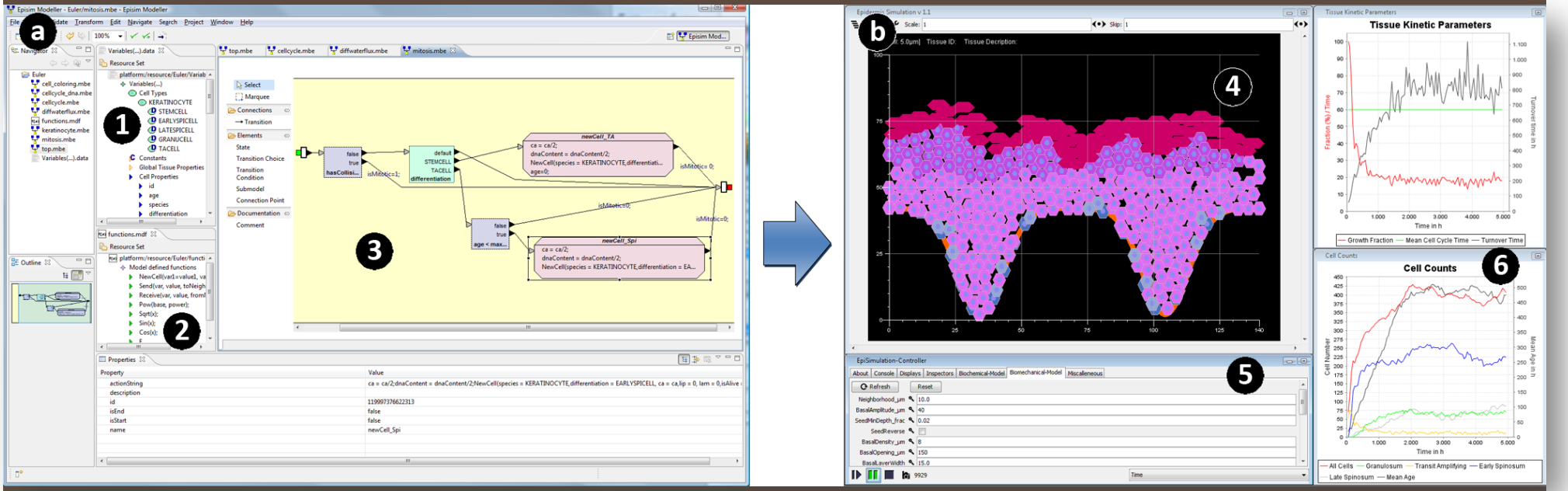
1. Systems biology drives towards tissue models
2. Quantitative spatial data of tissues are missing: why whole slide imaging is essential for systems biology of tissues
3. PART A: Generating data : Quantifying spatial protein expression data in human skin (Full slides & TMAs)
    - Application of quantitative spatial profiles for
        1. Reconstructing networks
        2. Building tissue classifiers
        3. Qualitative network reasoning
4. PART B: Generating data: Quantitative morphological analysis of dynamic wound healing via 3D in vitro cultures (Full slides)
    1. Analysis of cellular streams in tissues

„DRIVING THE SYSTEMS BIOLOGY OF TISSUES“

- So far mostly reductionist approach in biology:
  - Classic: Phenotype → Isolation of isolated genes → Function?
  - 2001: Sequencing of the human genome
  - Less genes than expected (24.000) → gene networks are essential
- Systems biological approach:
  - Quantitative and qualitative model building of cellular networks which can explain certain aspects of the functional behavior of a dynamic system
- *Tissue Systems Biology:*
  - Concerns building models of human tissue and diseases
  - Highly relevant for clinically oriented research
  - Requires spatial data (morphology, expression data)
  - Requires insights into mechanisms of spatial control

Nature 2001, **409:**860-921 und *Science* 2001, **291:**1304-1351

GRAPHICAL MODELLING SYSTEM

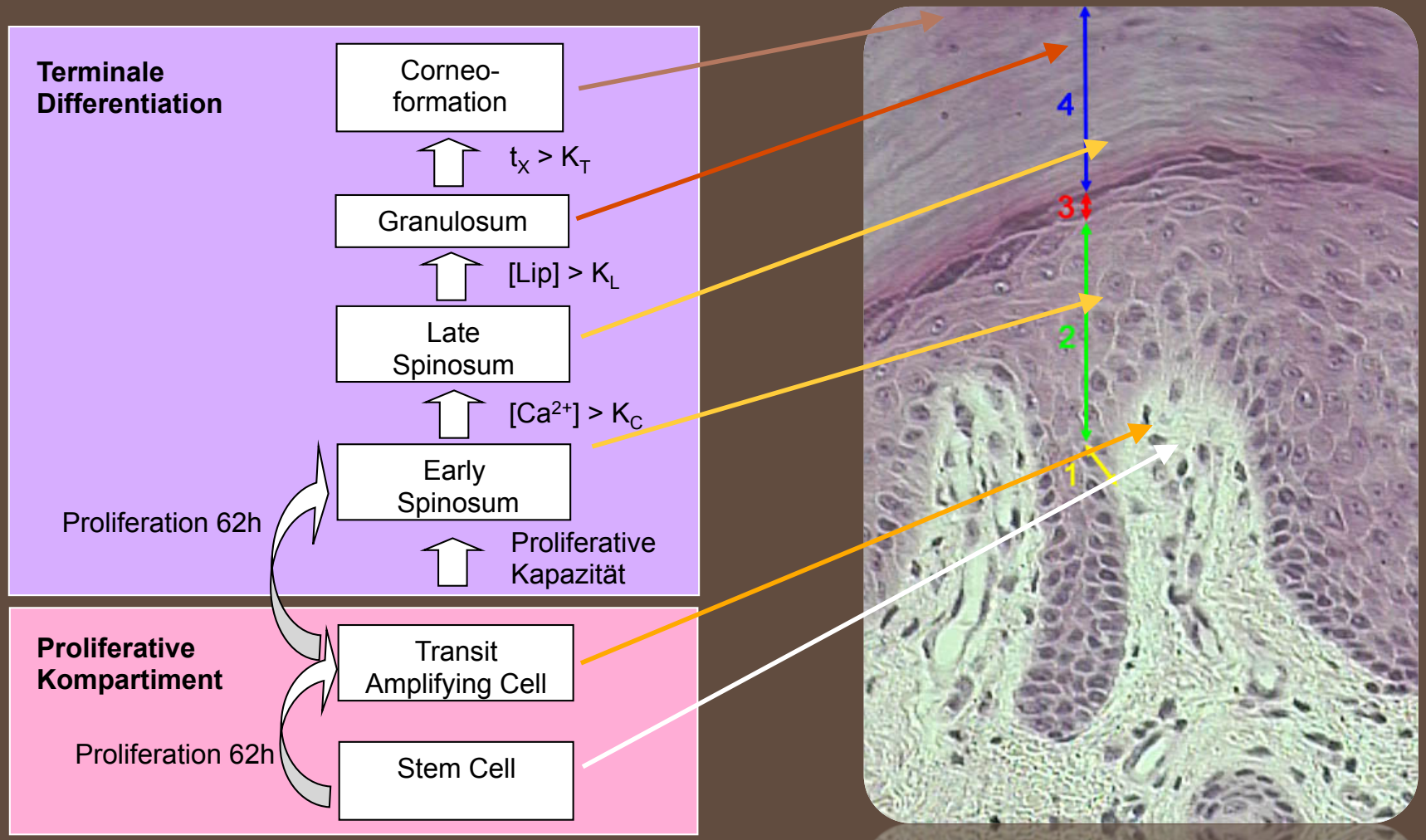


(1) Variable Sheet Editor, (2) Functions Library, (3) Graphical Model Editor

(4) Emerging Tissue Morphology, (5) Parameter Window, (6) Dynamically generated graphs

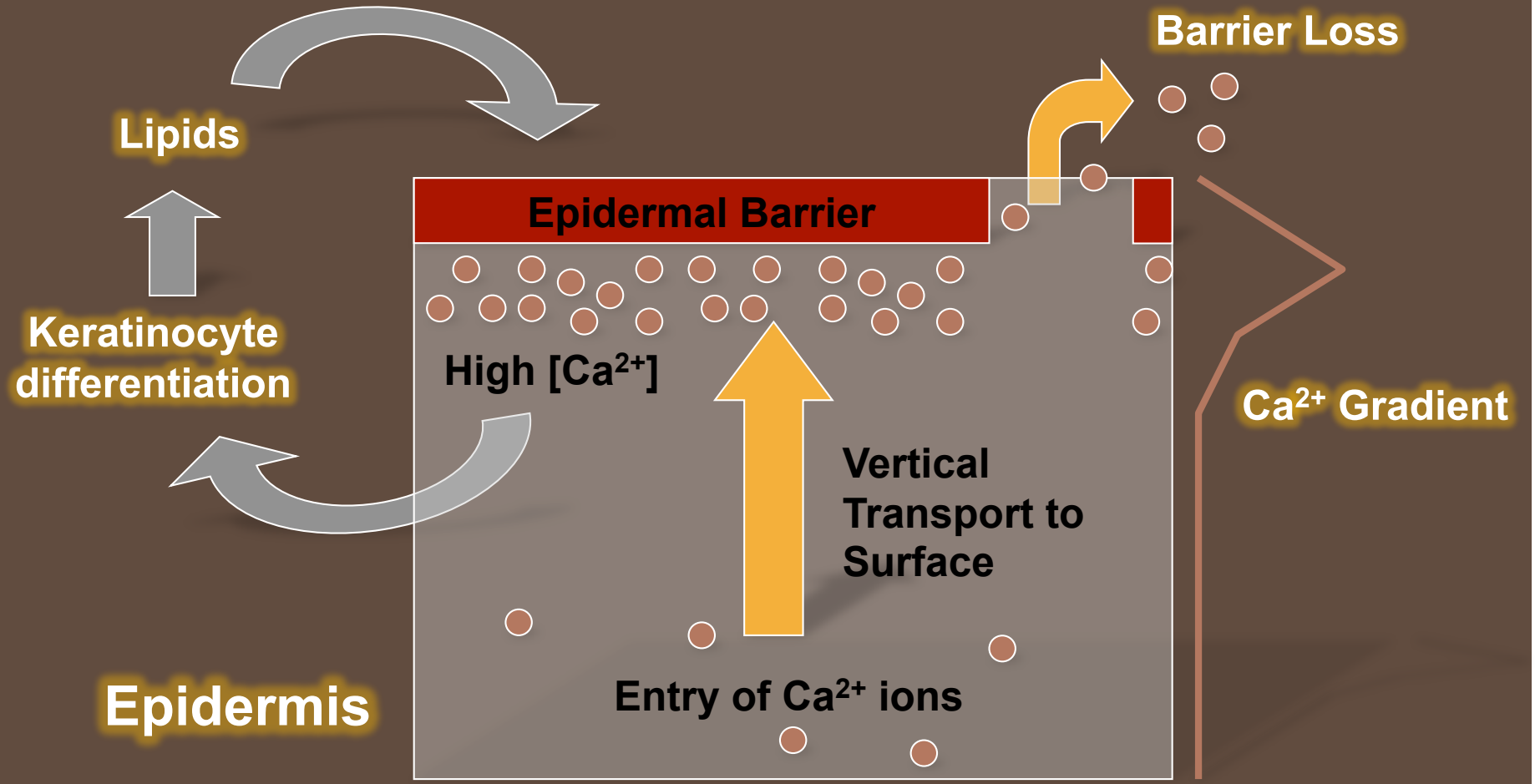
Sütterlin T, Huber S, Dickhaus H, Grabe, N.
Modeling multi-cellular behavior in epidermal tissue homeostasis via finite state machines in multi-agent systems *Bioinformatics* (2009), 25, 2057-2063.

STATE BASED SPATIAL MODELING OF CELL DIFFERENTIATION



N. Grabe, K. Neuber. A multi-cellular systems biology model predicts epidermal morphology, kinetics, and Ca++ flow. *Bioinformatics* (2005) Sep 1;21(17):3541-7

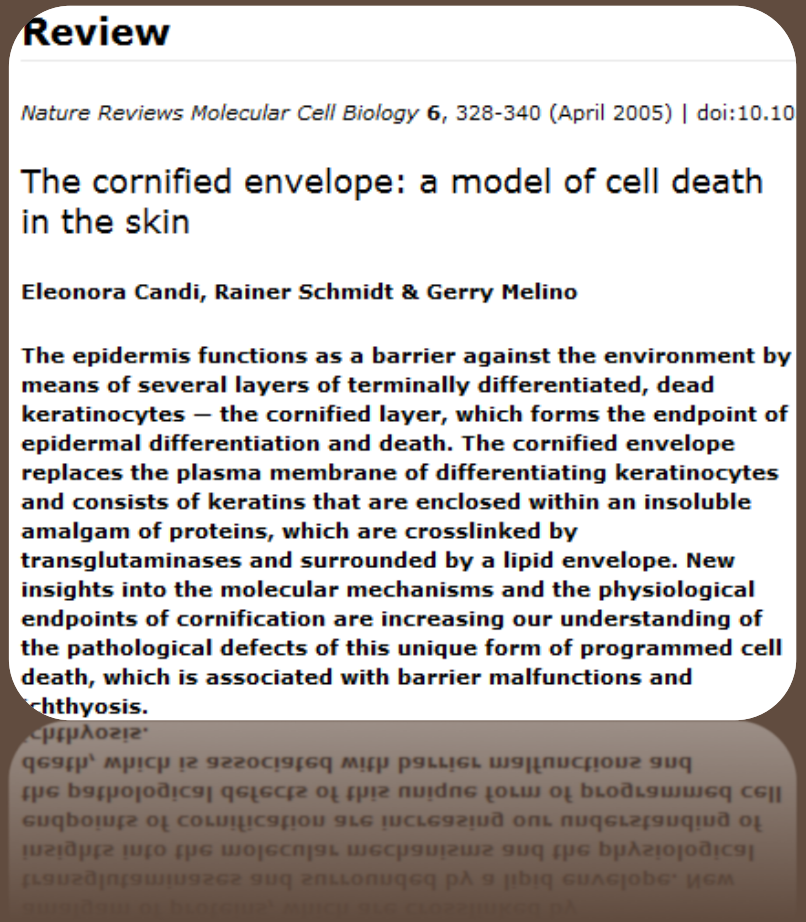
WHAT DRIVES THE MODEL ?
EMERGENCE OF THE CALCIUM GRADIENT



Grabe N, Neuber K.
A multicellular systems biology model predicts epidermal morphology, kinetics and Ca2+ flow , *Bioinformatics*. 2005 Sep 1;21(17):3541-7.

WHERE ARE THE SPATIAL DATA??

- Simulation proof of principle shown, but:
- Two key questions:
  - How are genes & proteins spatially expressed in the skin?
  - Why are they expressed the way they are?
- What do we know?
  - 20041 publications in pubmed („epidermis human skin“)
  - Buried in pubmed? „Partly yes“ but:
  - No quantitative spatial expression data for skin available !
  - No networks are available !



Review

Nature Reviews Molecular Cell Biology **6**, 328-340 (April 2005) | doi:10.10

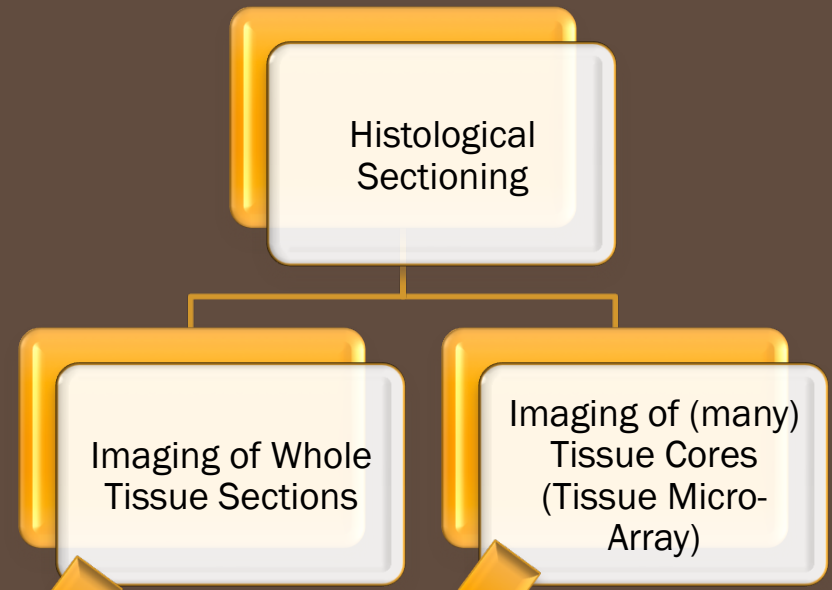
The cornified envelope: a model of cell death in the skin

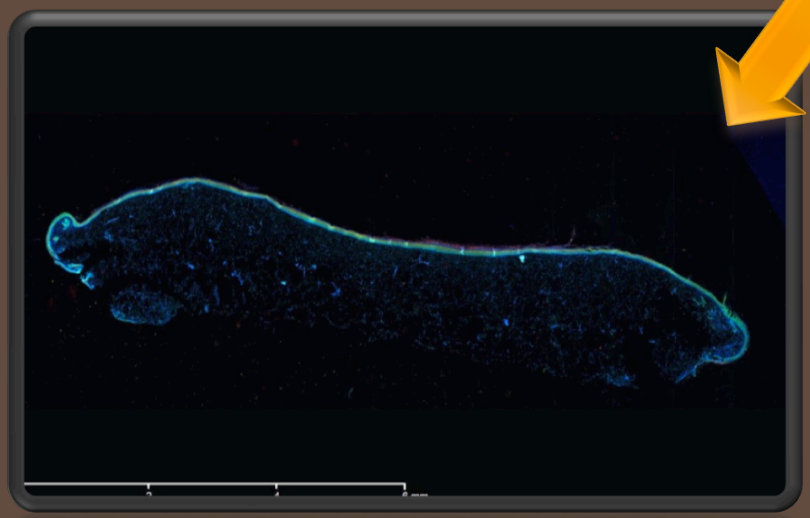
Eleonora Candi, Rainer Schmidt & Gerry Melino

The epidermis functions as a barrier against the environment by means of several layers of terminally differentiated, dead keratinocytes — the cornified layer, which forms the endpoint of epidermal differentiation and death. The cornified envelope replaces the plasma membrane of differentiating keratinocytes and consists of keratins that are enclosed within an insoluble amalgam of proteins, which are crosslinked by transglutaminases and surrounded by a lipid envelope. New insights into the molecular mechanisms and the physiological endpoints of cornification are increasing our understanding of the pathological defects of this unique form of programmed cell death, which is associated with barrier malfunctions and ichthyosis.

AUTOMATIC WHOLE SLIDE IMAGING (WSI) = VIRTUAL MICROSCOPY (VM), SLIDE SCANNING

230 nm resolution, 40 GB uncompressed

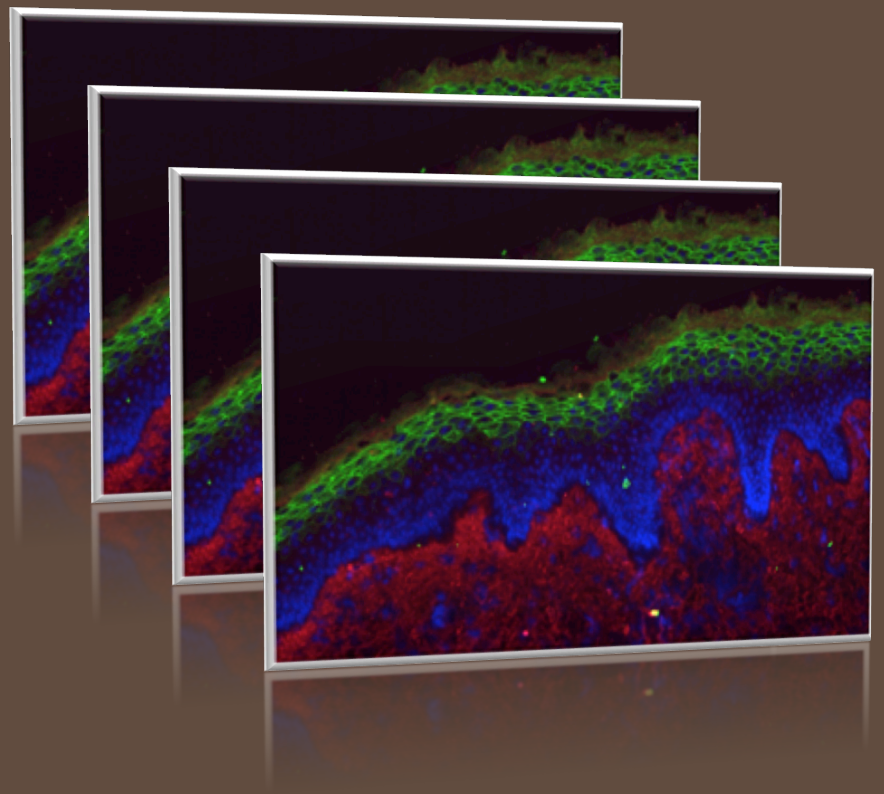


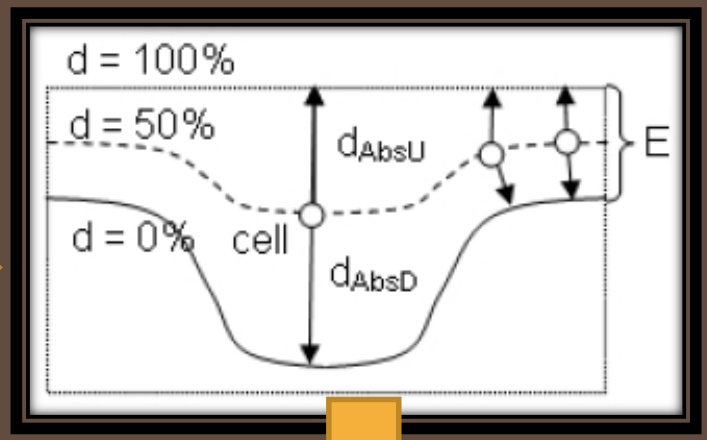


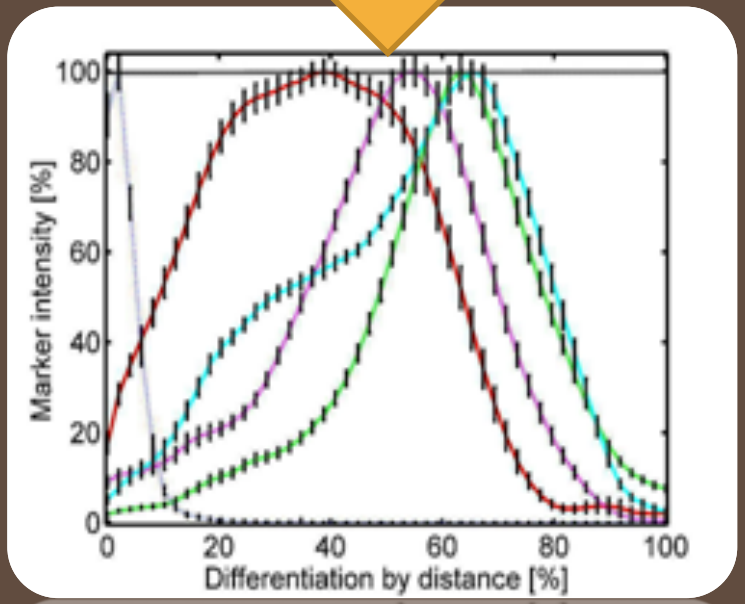
PART A

- Generating data : Quantifying spatial protein expression data in human skin (Full slides & TMAs)
    1. Reconstructing networks
    2. Building tissue classifiers
    3. Qualitative network reasoning

QUANTITATIVE SPATIAL PROFILES (QSPS)

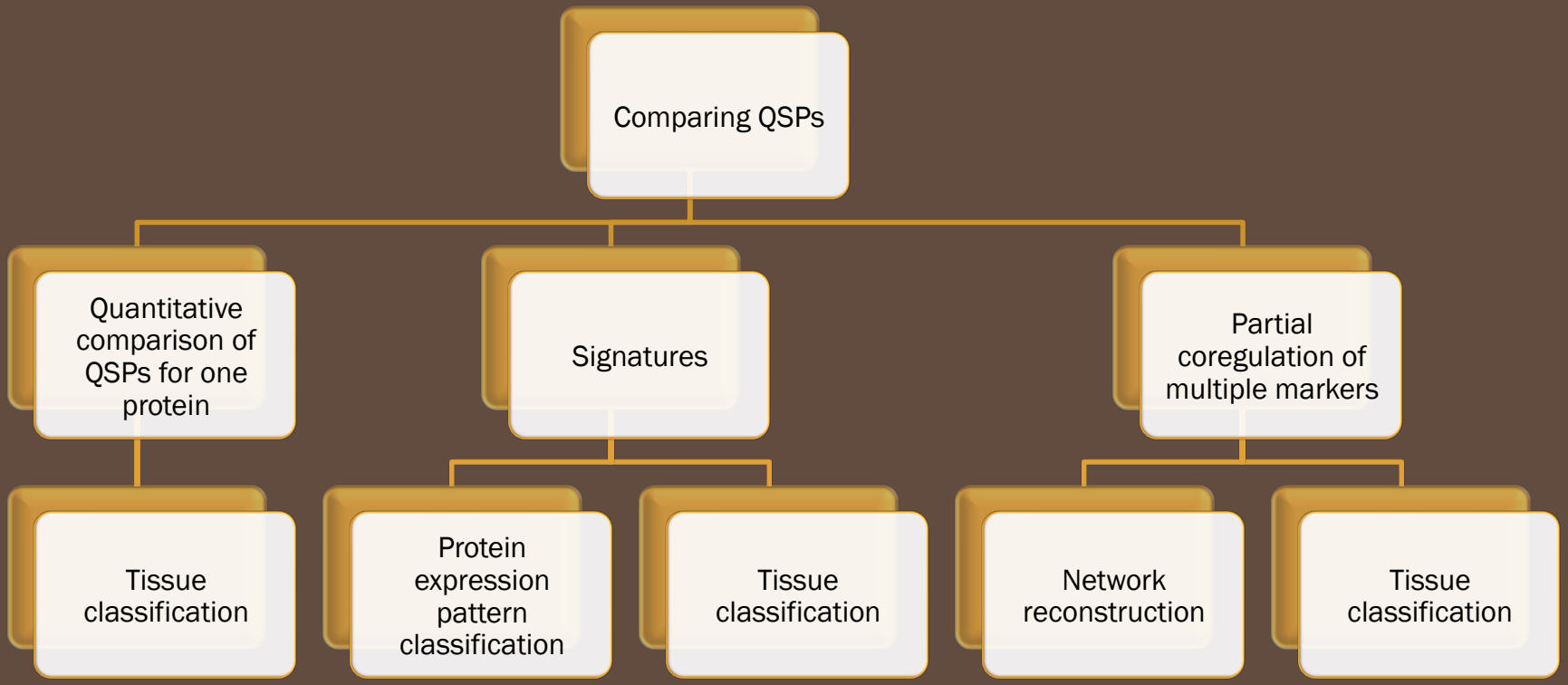




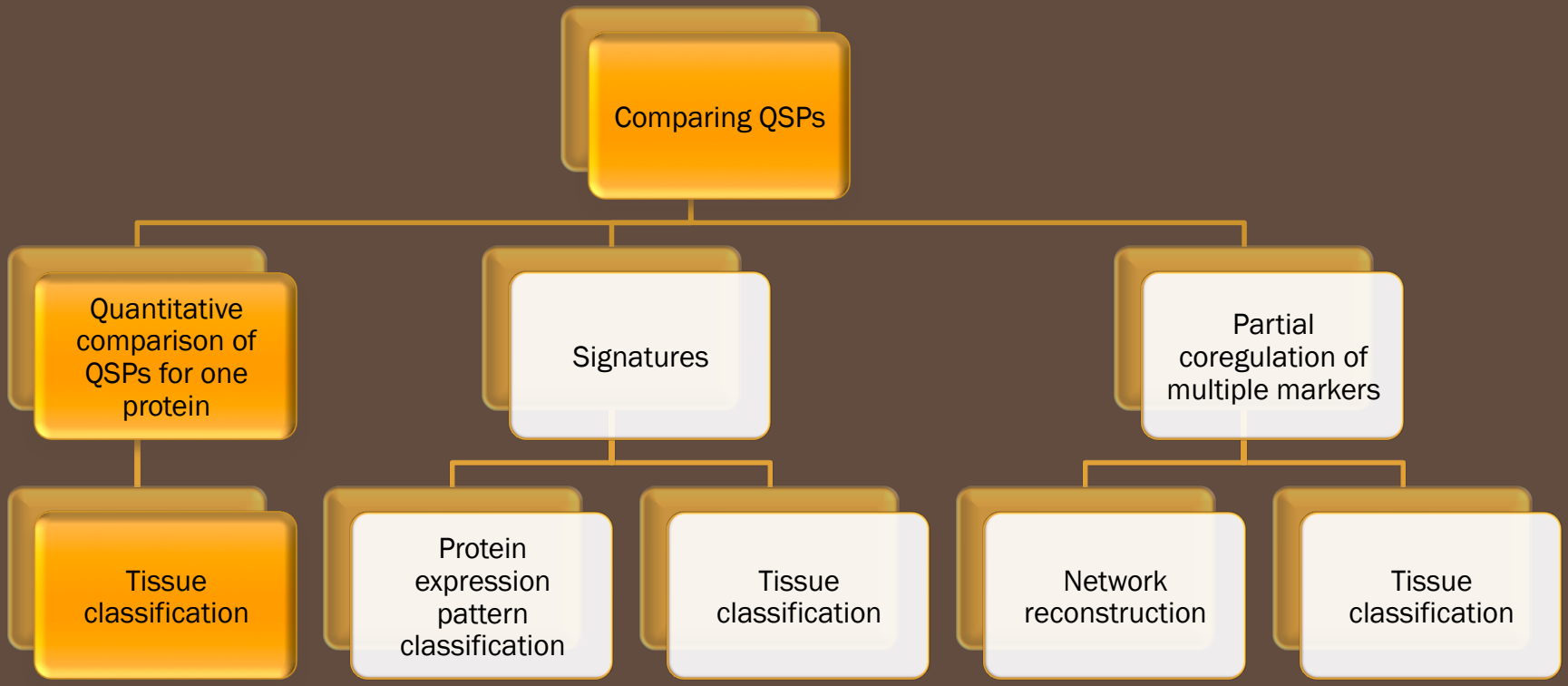


Grabe, Pommerencke, Steinberg, Dickhaus, Tomakidi, *Reconstructing Protein Networks of Epithelial Homeostasis* , Bioinformatics, 2007

APPLICATIONS OF EPIDERMAL QUANTITATIVE SPATIAL PROFILING

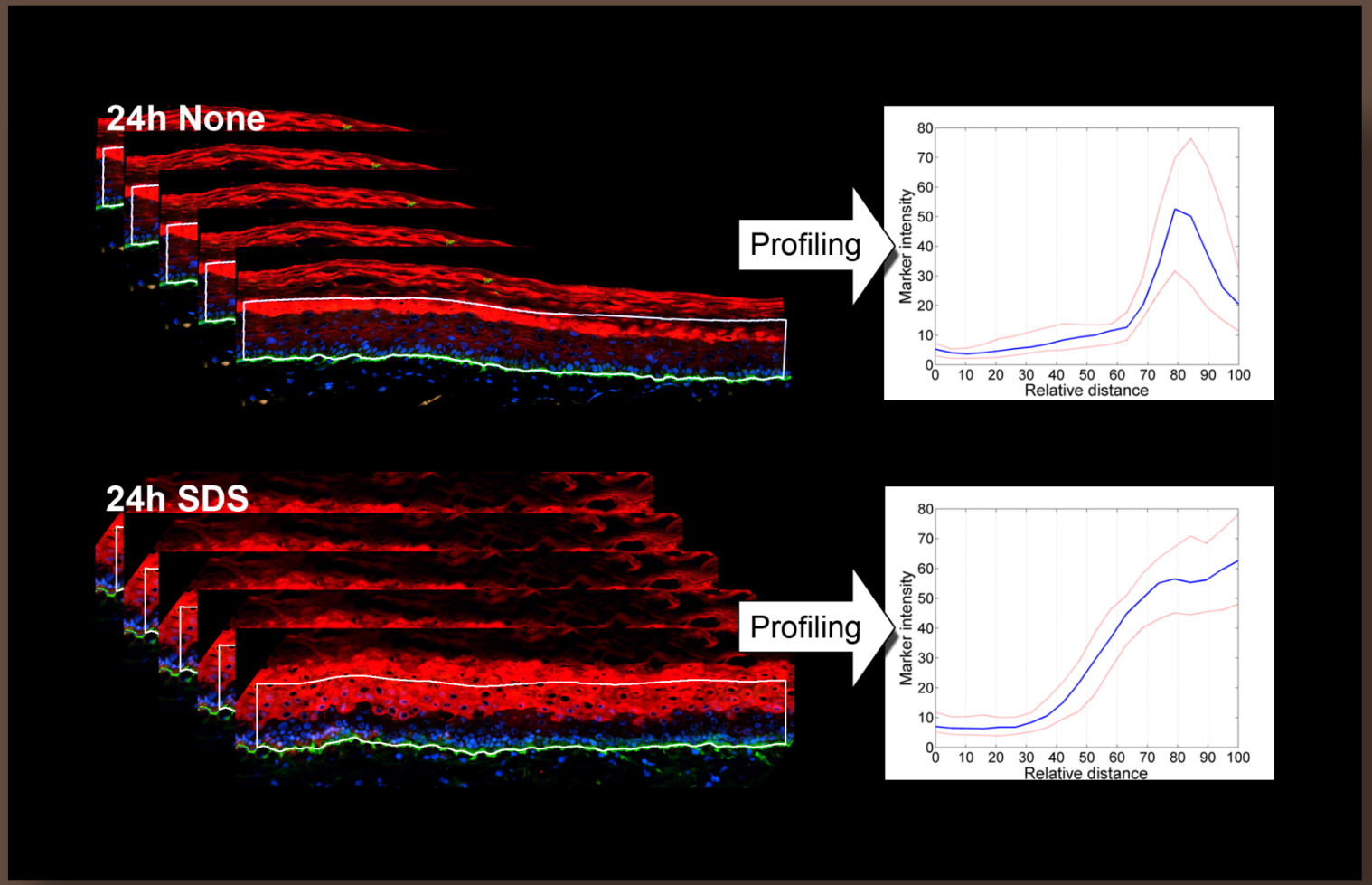


APPLICATIONS OF EPIDERMAL QUANTITATIVE SPATIAL PROFILING

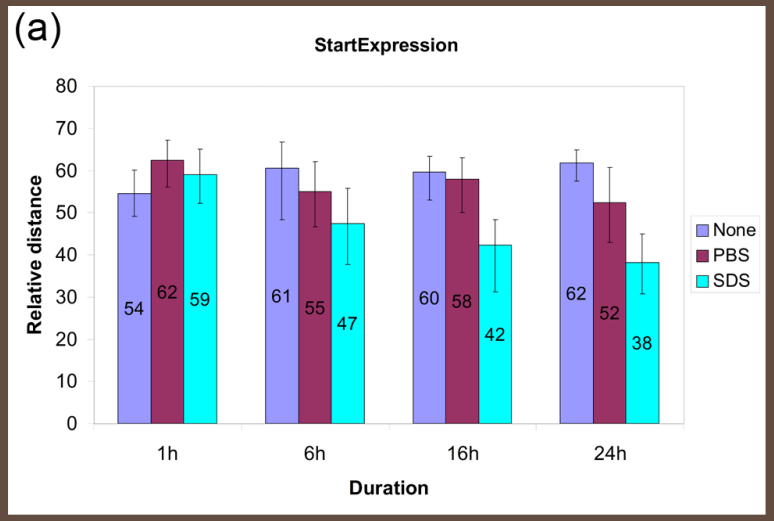


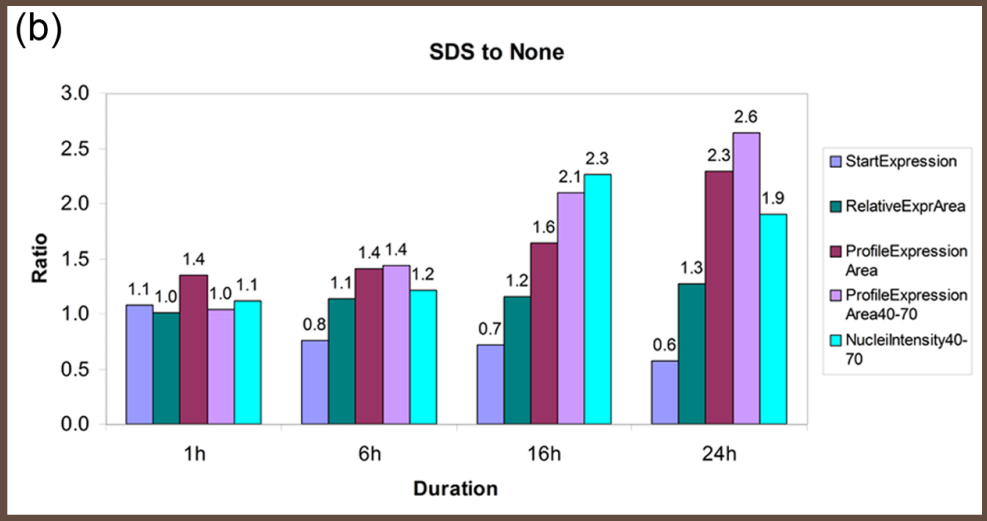
IRRITATION PREDICTION BY IMAGING OF HSP27

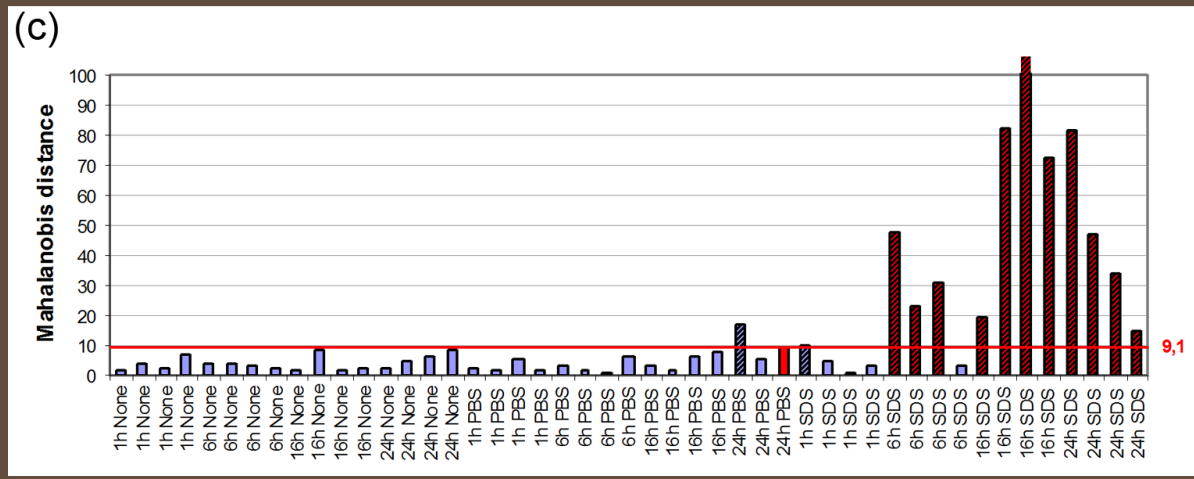
- Mattek EFT Cultures treated with 0.4% SDS for 1h, 6h, 16h, 24h
- Profiling of Heat Shock Protein HSP27

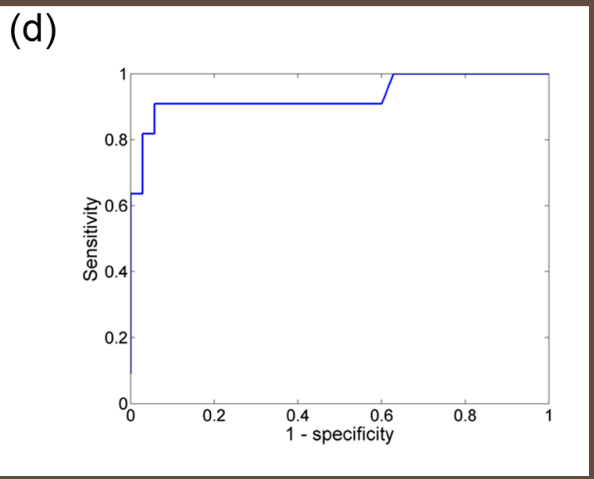


CLASSIFICATION OF SKIN IRRITATION

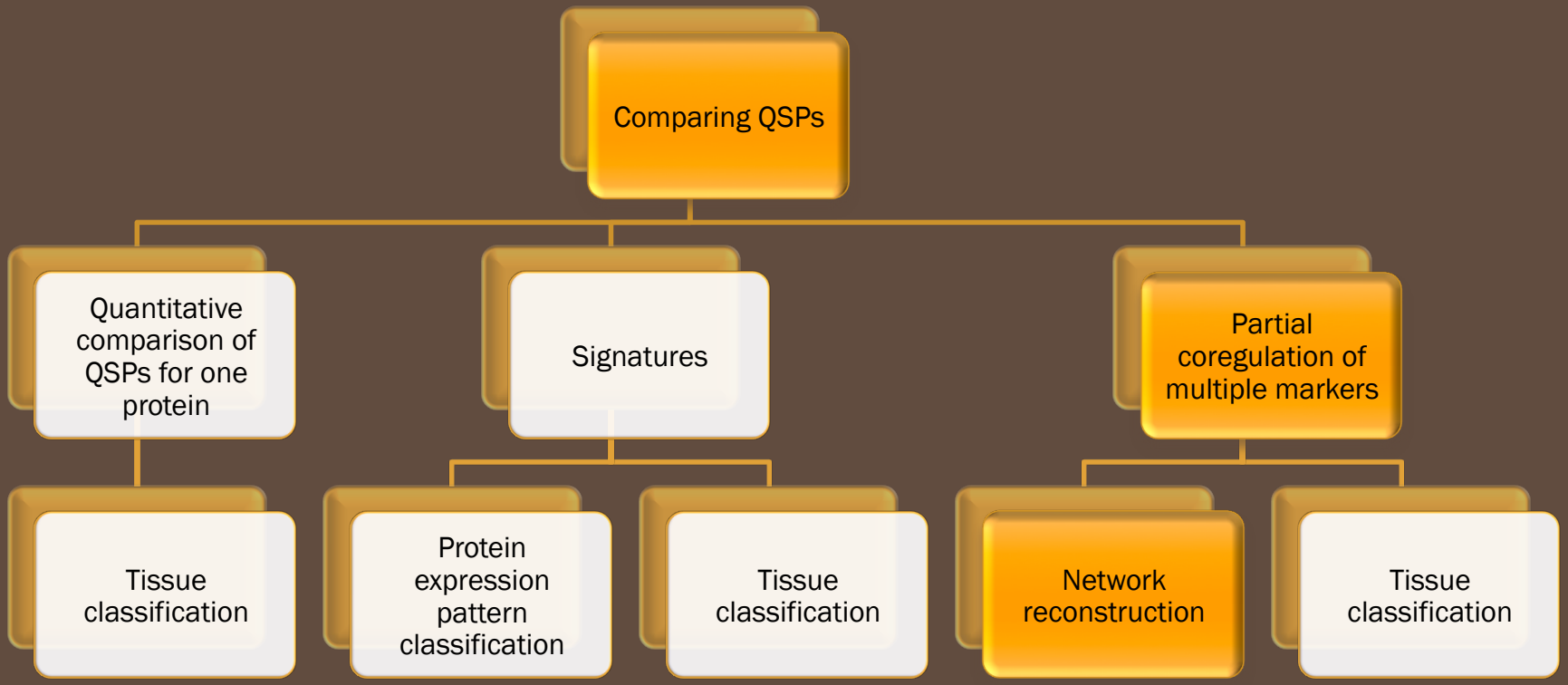




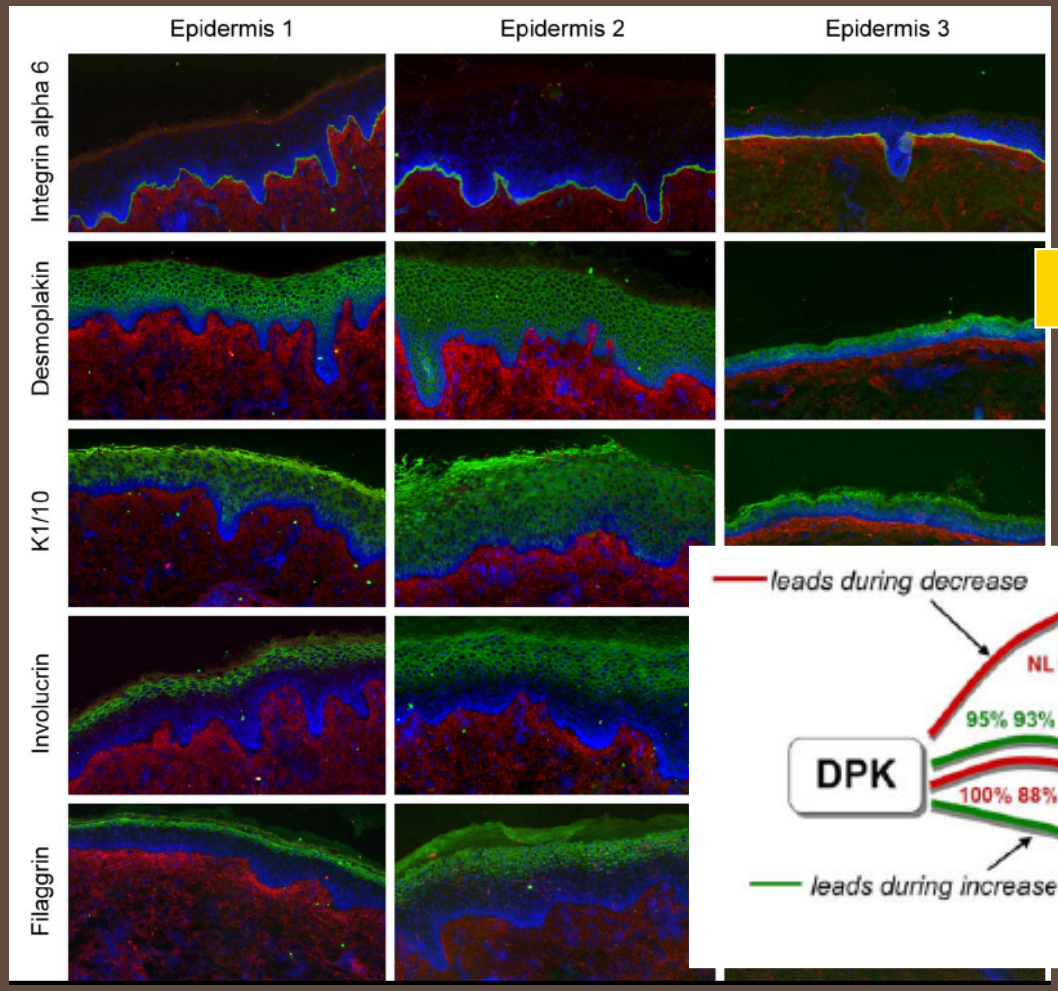




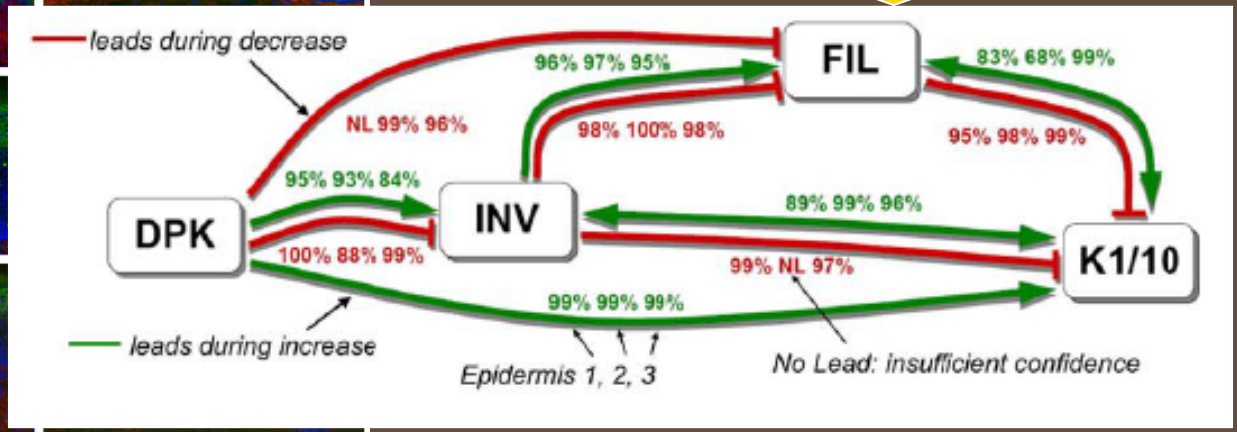
APPLICATIONS OF EPIDERMAL QUANTITATIVE SPATIAL PROFILING



RECONSTRUCTING PROTEIN NETWORKS OF EPIDERMAL DIFFERENTIATION

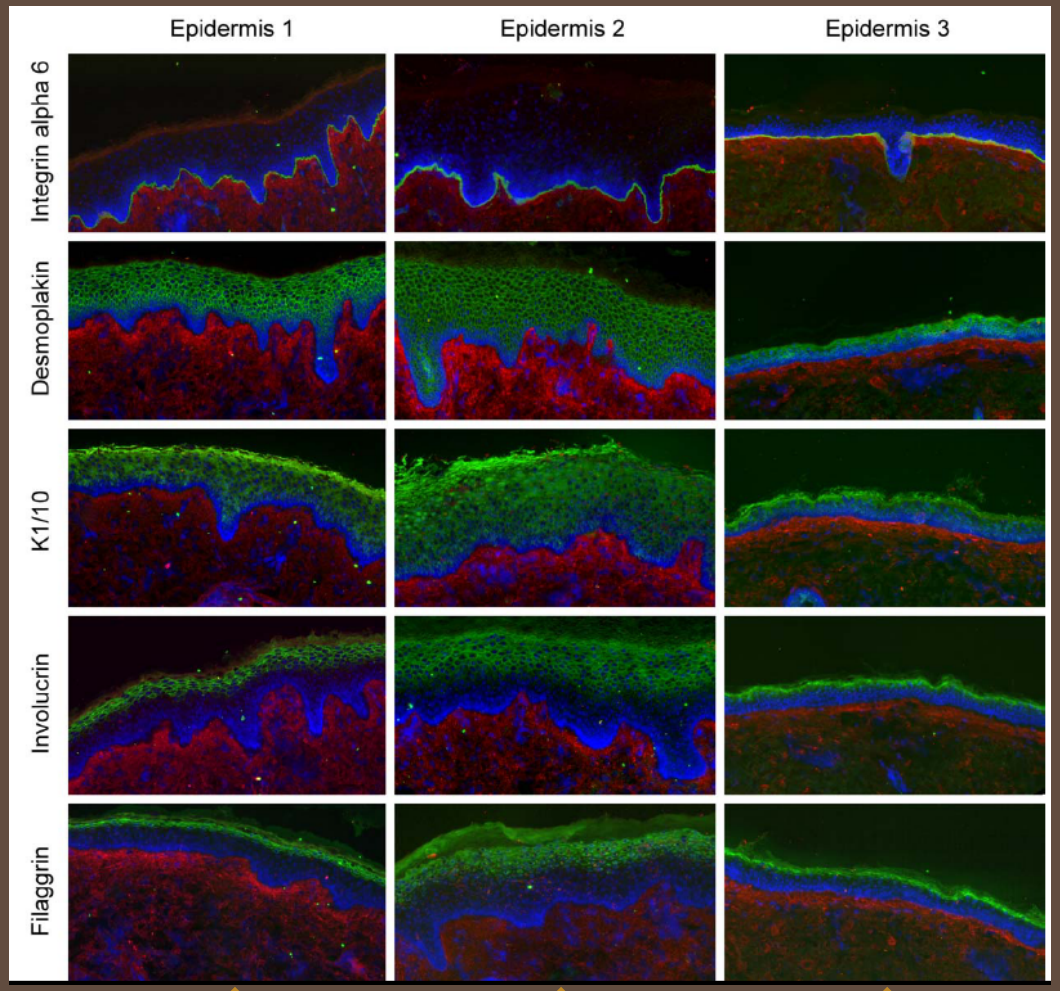


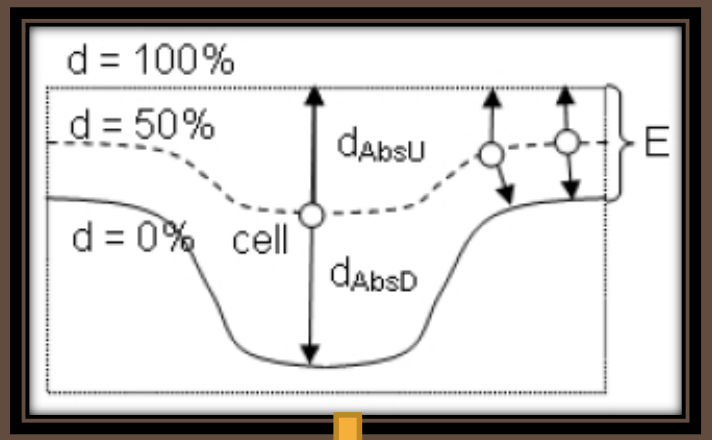
1. Microscopic Imaging
2. Image Processing
3. Quantitative Biomarker Profiling
4. Network Reconstruction



Grabe, Pommerencke, Steinberg, Dickhaus, Tomakidi, *Reconstructing Protein Networks of Epithelial Homeostasis* , Bioinformatics, 2007

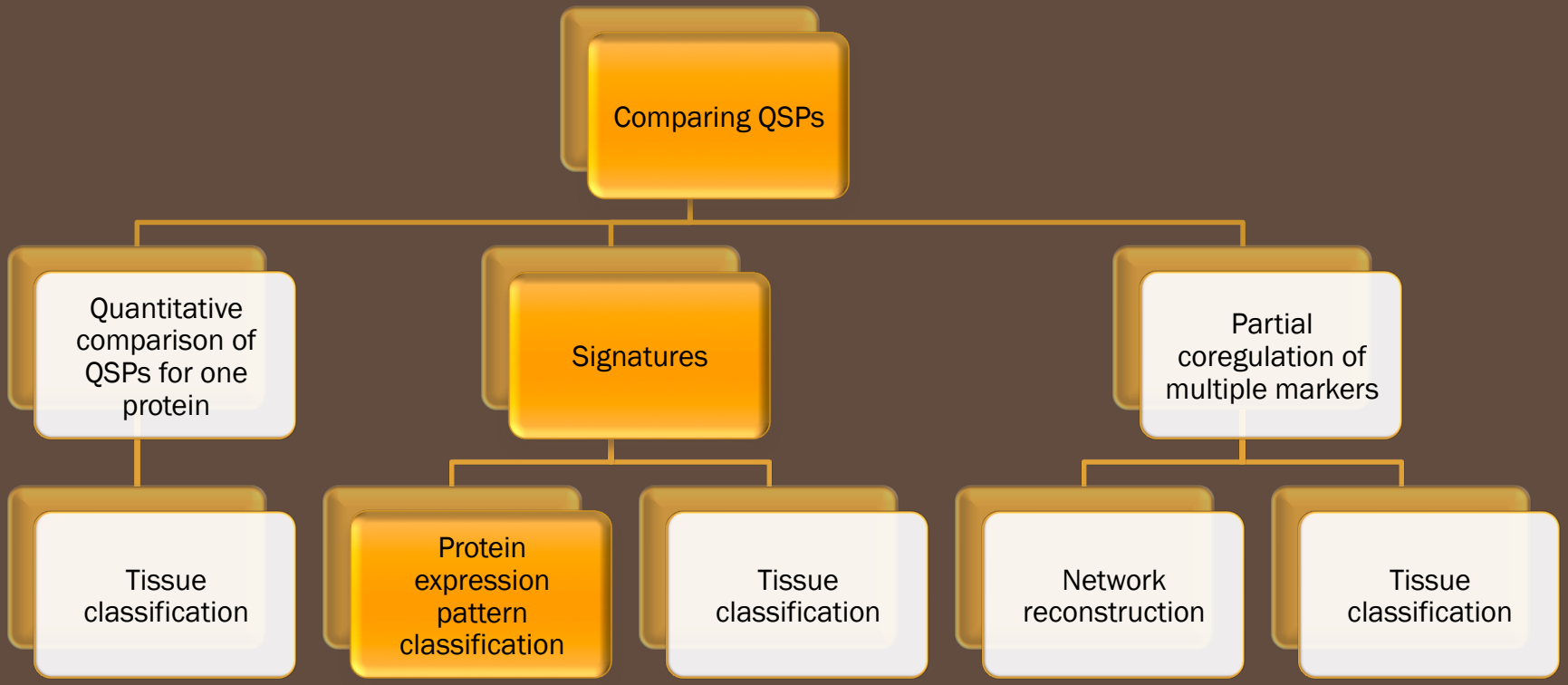
EXISTENCE OF ROBUST MECHANISMS FOR SPATIAL REGULATION OF DIFFERENTIATION





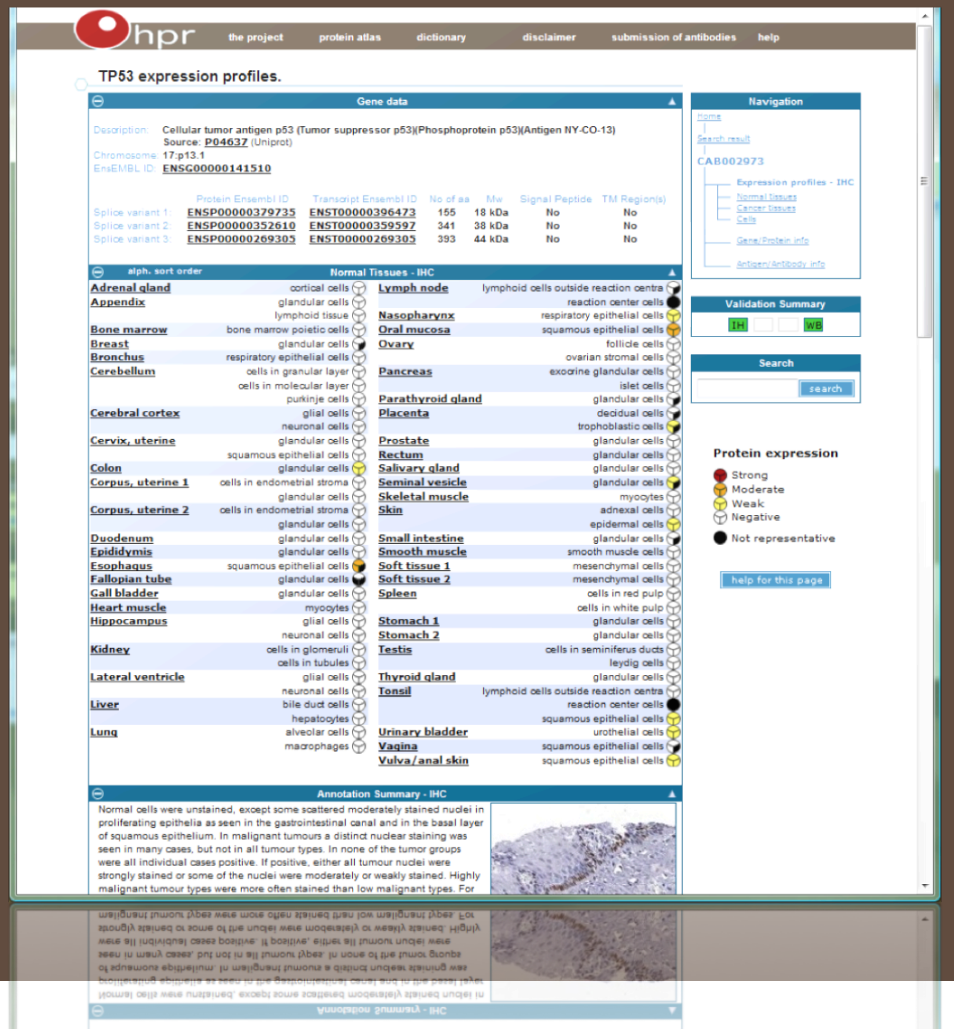
Which mechanisms regulate differentiation in time and space?

APPLICATIONS OF EPIDERMAL QUANTITATIVE SPATIAL PROFILING



HUMAN PROTEIN ATLAS

- Largest Knowledge Ressource on Spatial Protein Expression Patterns
- 8832 antibodies against all types of human tissue
- 7,334,244 images of tissue microarrays (TMA)



CAN THE HPA USED TO GENERATE QSPS?

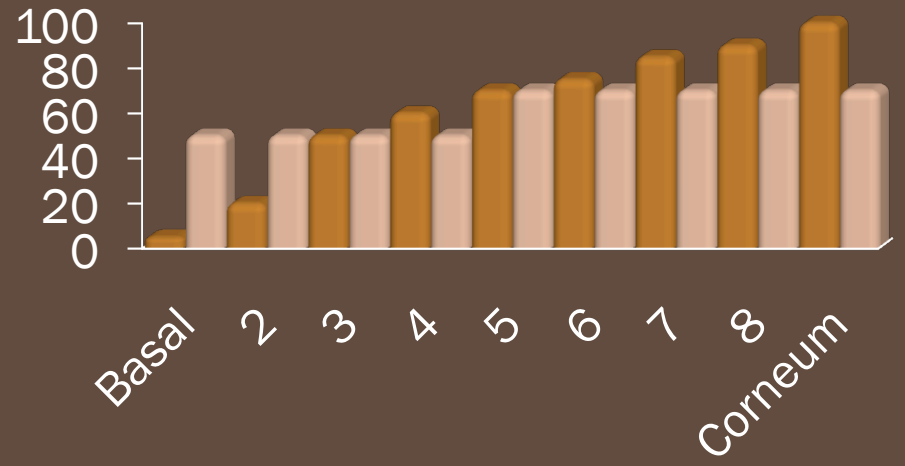
- Skin tissue sections are „just“ Tissue Microarrays and no whole slide scans
- => No, it cannot generate the required data:
  - No fluorescent staining => image segmentation automatically not with sufficient quality
  - Too few sections => intra-tissue variation too high
- What could HPA data be used for?
  - Not direct comparison of QSPs but abstraction of QSPs in the form of Signatures
- Technically challenging: combination of QSP approach with color unmixing (→Robert Murphy)

SPATIAL SIGNATURES

Basal Signature

Intermediate Signature

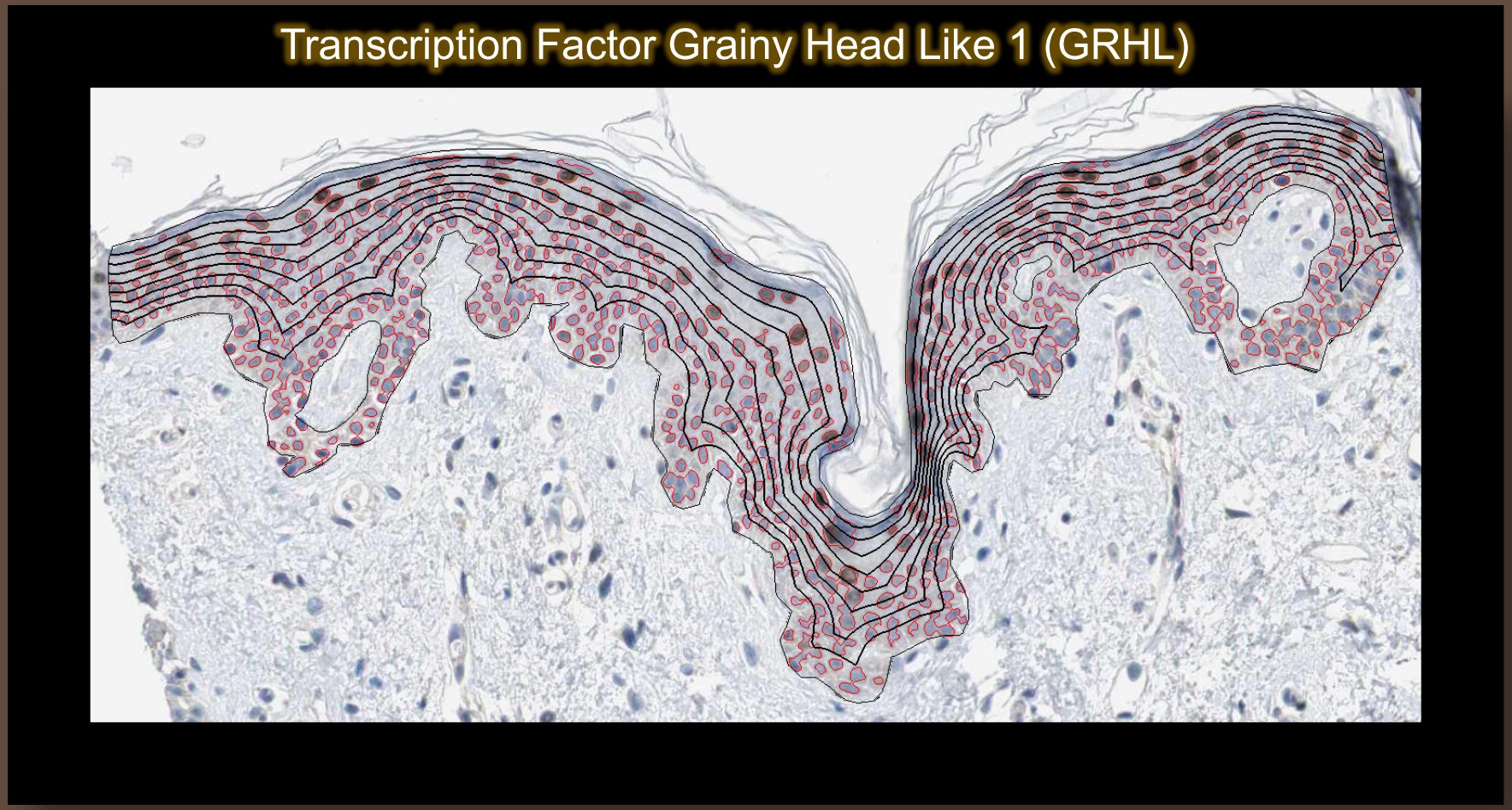
Gradient Signature



Top Signature

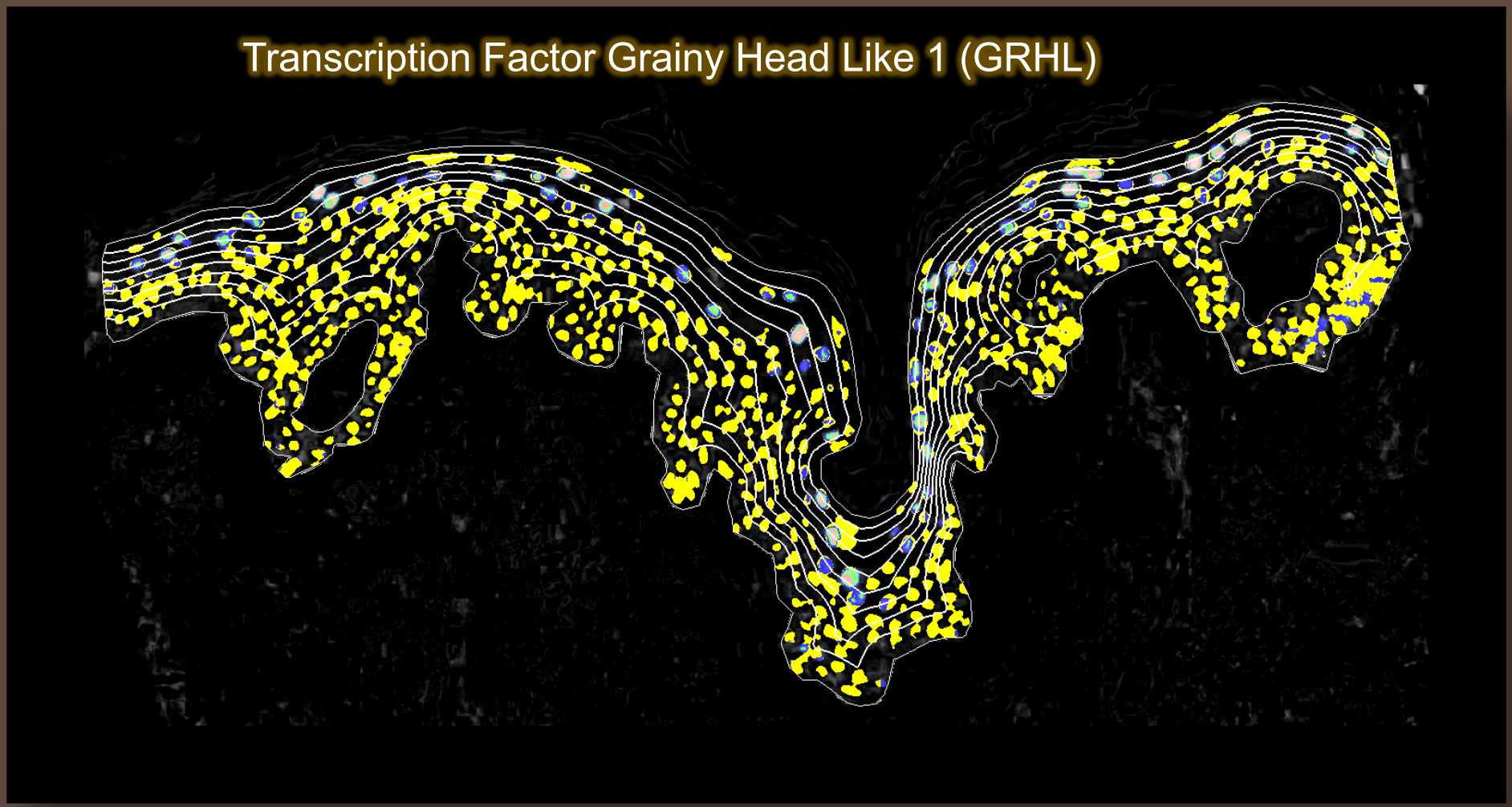
NUCLEI DETECTION

- Epidermal Differentiation in 9 Layers

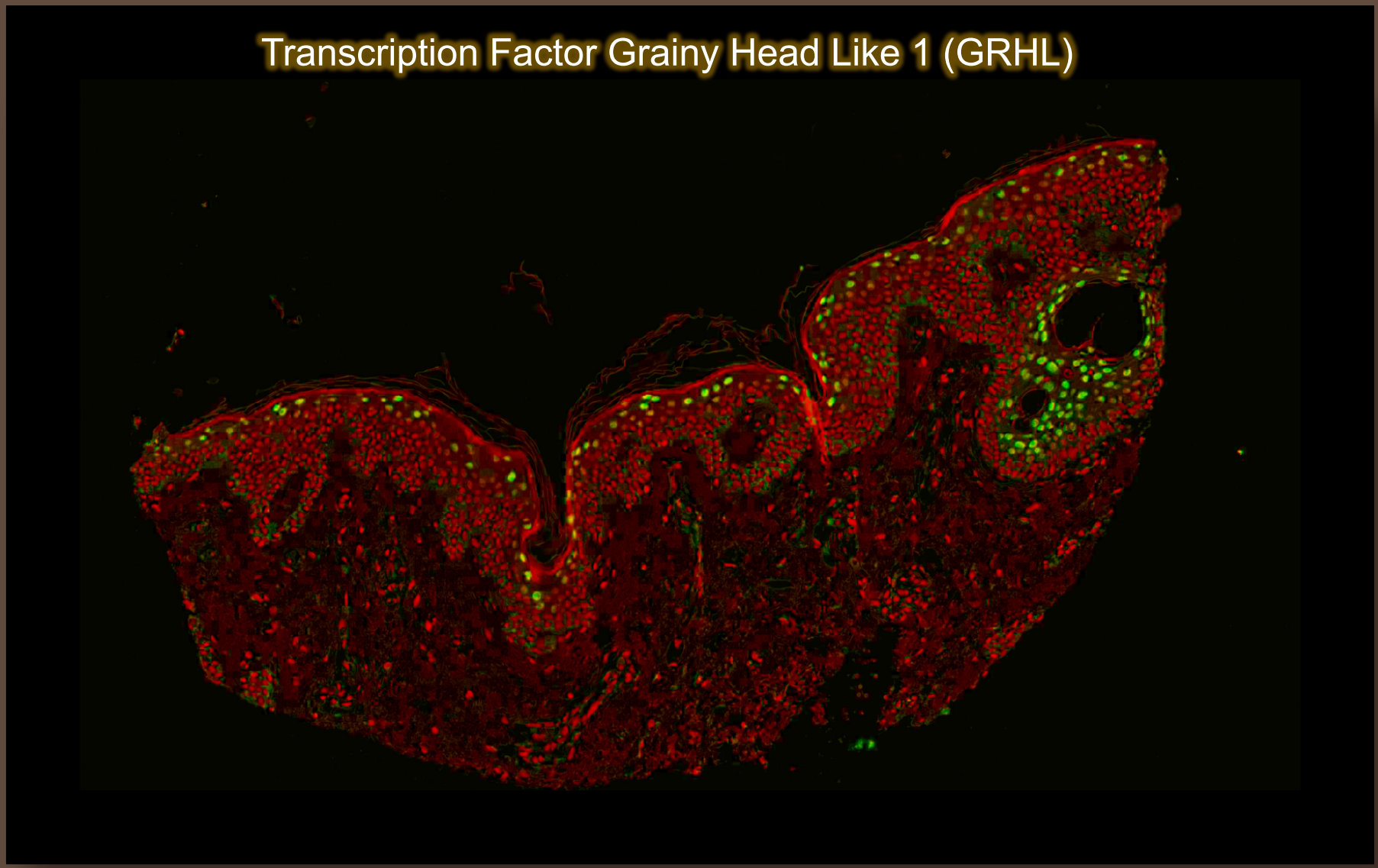


NUCLEI DETECTION & QUANTIFICATION

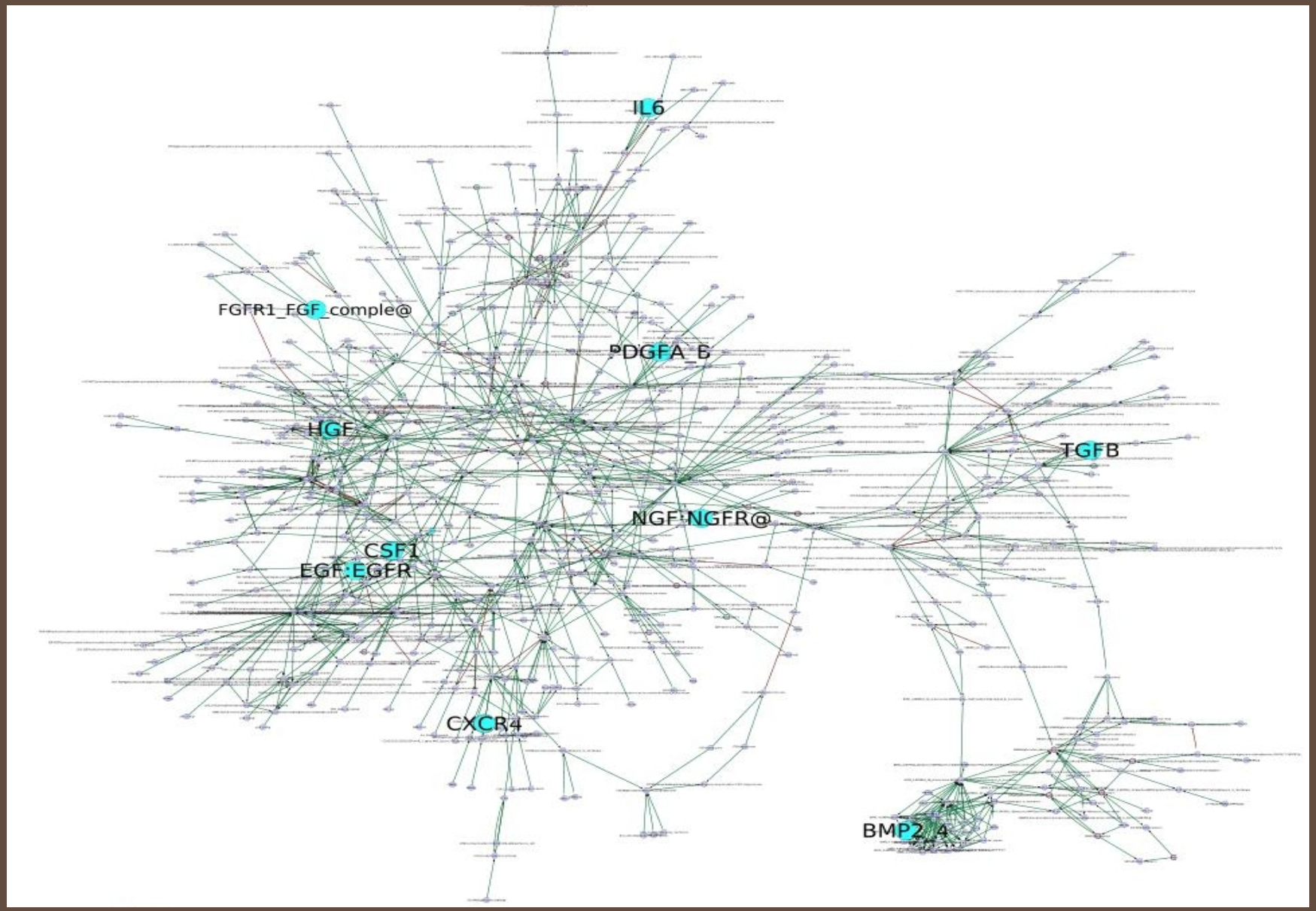
- Bands overlayed with staining intensities



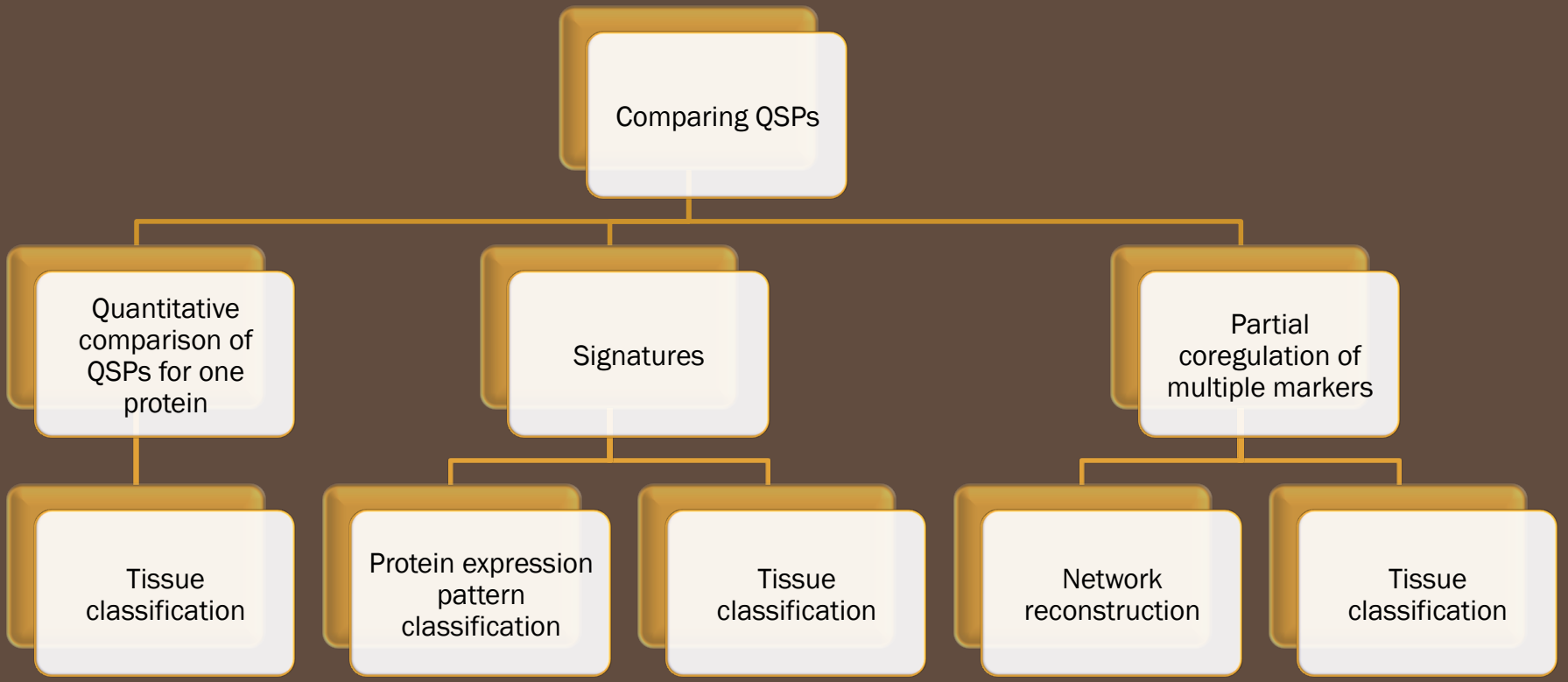
COLOR DECOMPOSED DAB/HEMALAUN STAIN



LARGE SCALE NETWORK OF KEY GROWTH FACTORS IN EPIDERMAL WOUND HEALING



APPLICATIONS OF EPIDERMAL QUANTITATIVE SPATIAL PROFILING



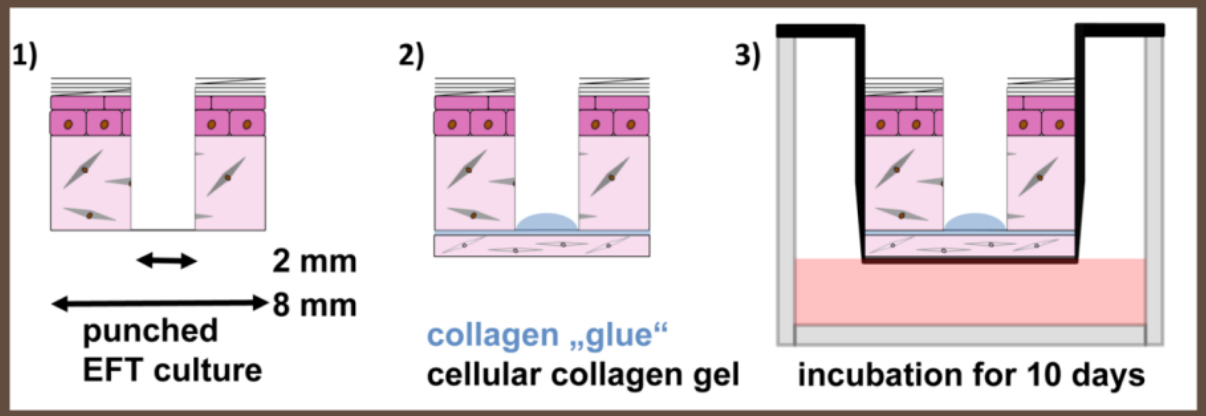
Enables =>
Correlative Analysis of Pathways in Keratinocyte Differentiation

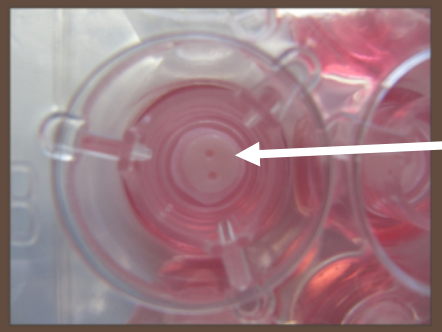
PART B

1. PART B: Generating data: Quantitative morphological analysis of dynamic wound healing (Full slides)
    1. Analysis of cellular streams in tissues

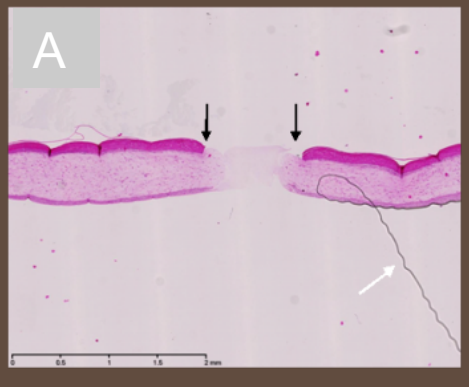
A NEW STANDARDIZED SKIN PUNCH MODEL

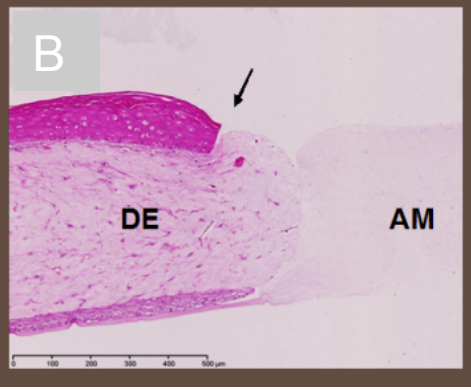
- Built on basis of a commercial skin culture system (Mattek)
- Dermis (Fibroblasts) + Epidermis (Keratinocytes)
- Full stratification (all differentiation stages)
- Reproducible(+), availability (+), costs (-), grown-up already (-)

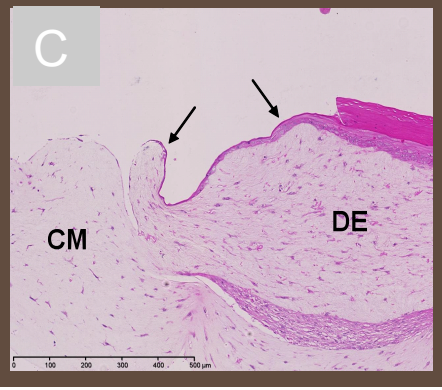


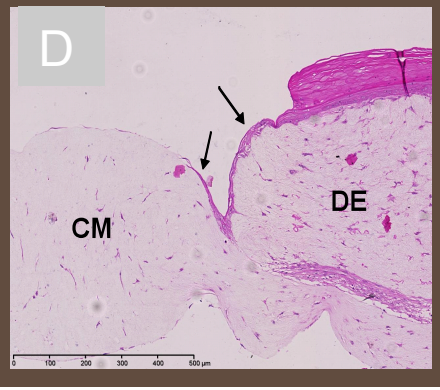


Punched Culture

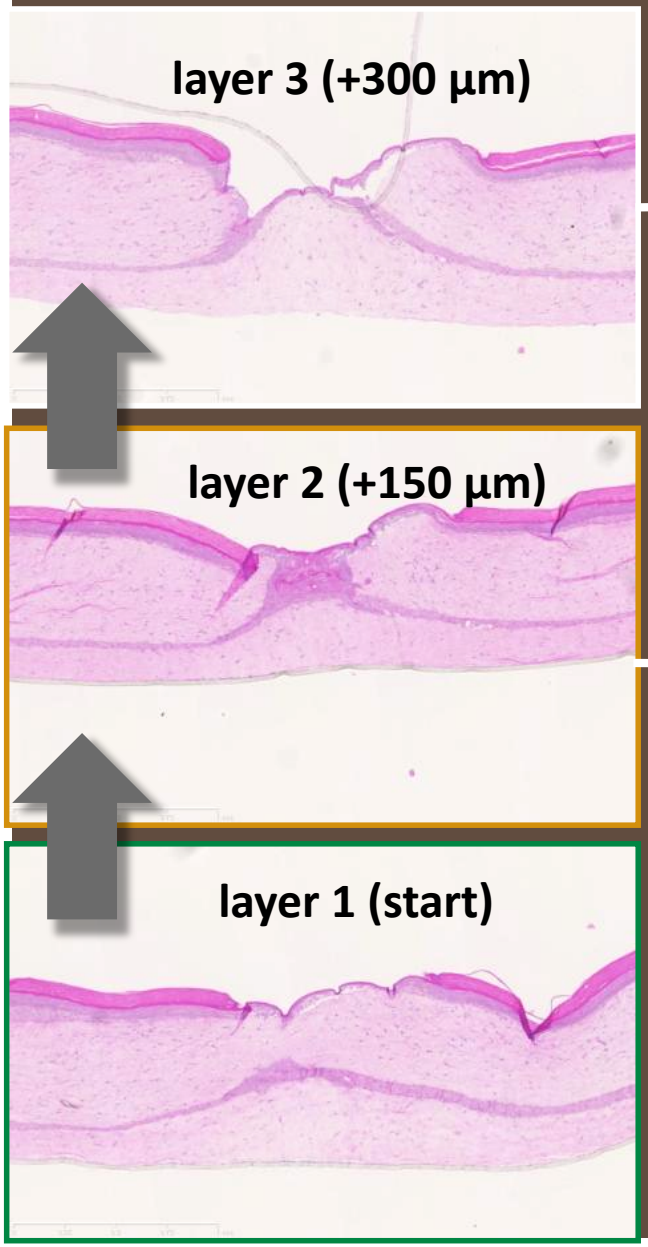


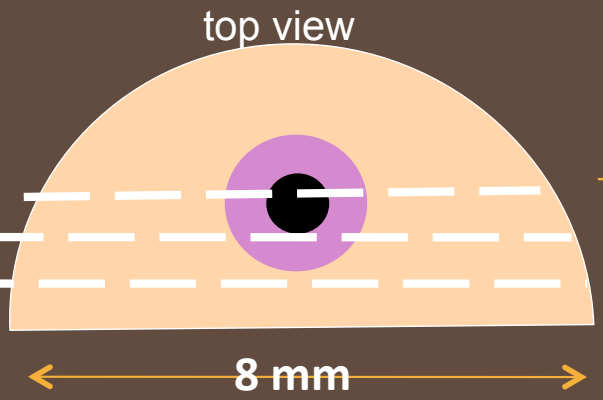






SYSTEMATIC HISTOLOGICAL ANALYSIS

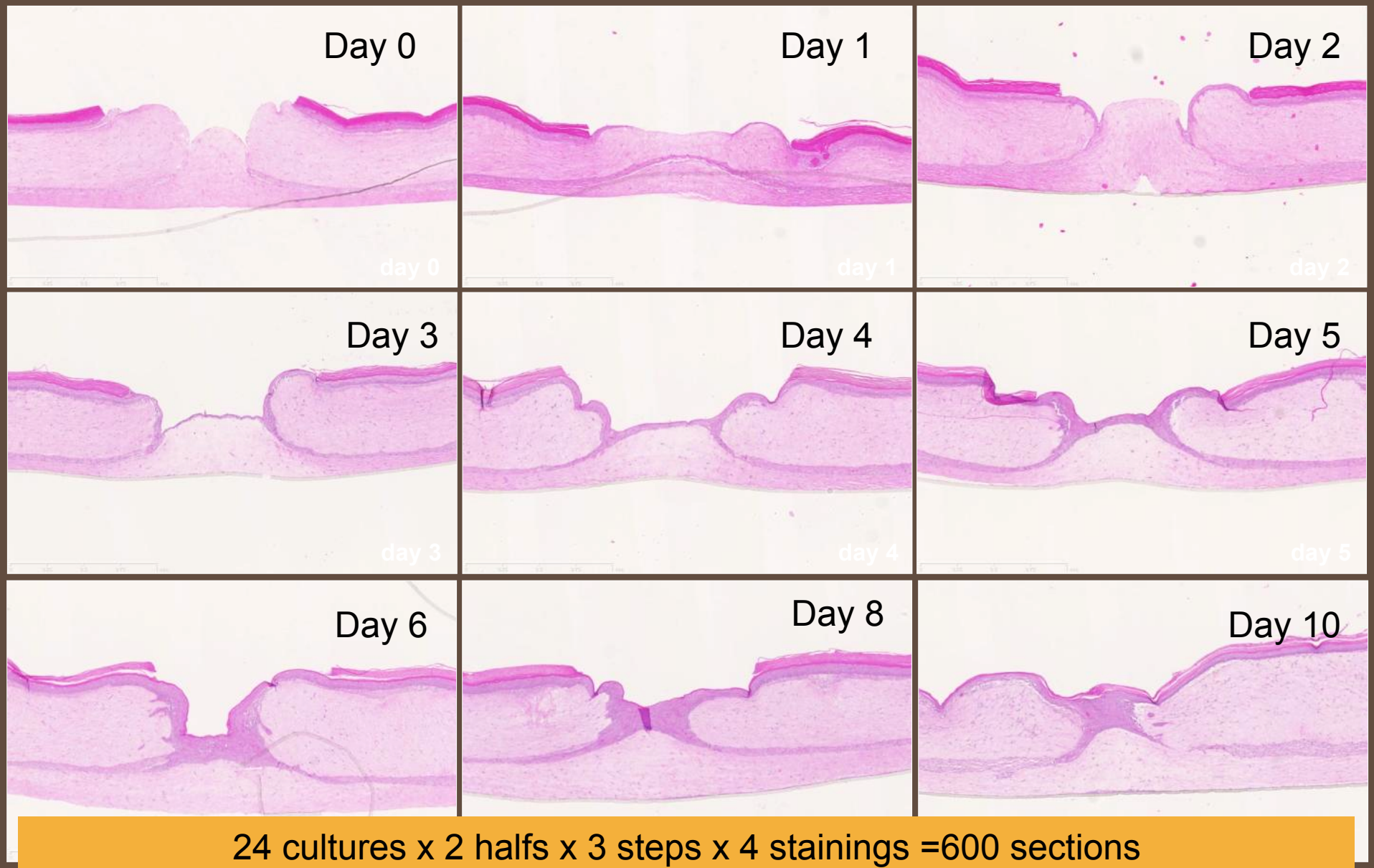




Autostainer with standardized staining protocols

Automatic microscopic Whole Slide Imaging (WSI)

WOUND CLOSURE IN NOVEL IN VITRO FULL SKIN MODEL - DAY 0 TO DAY 10 (24 TISSUES)

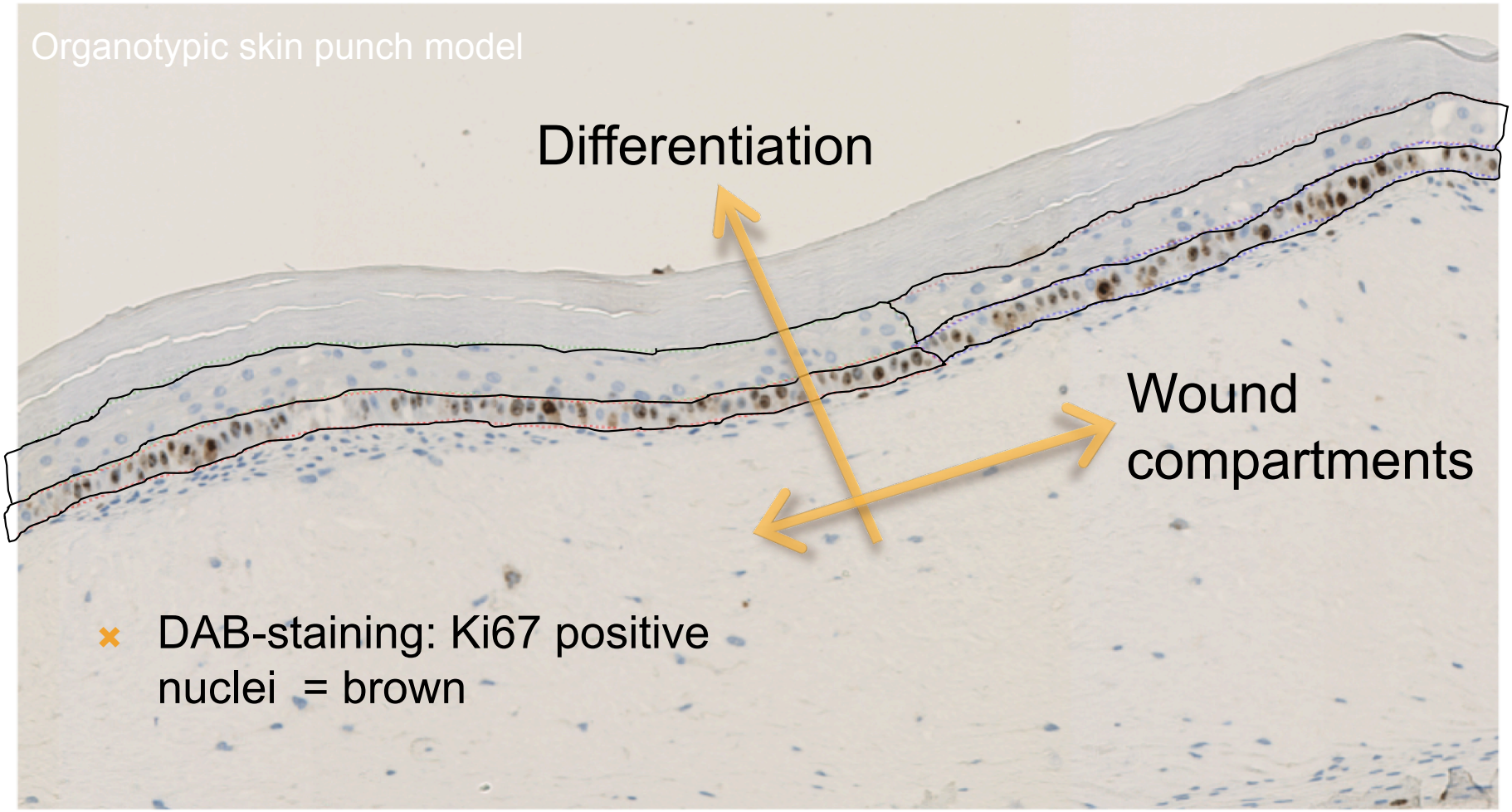


24 cultures x 2 halfs x 3 steps x 4 stainings =600 sections

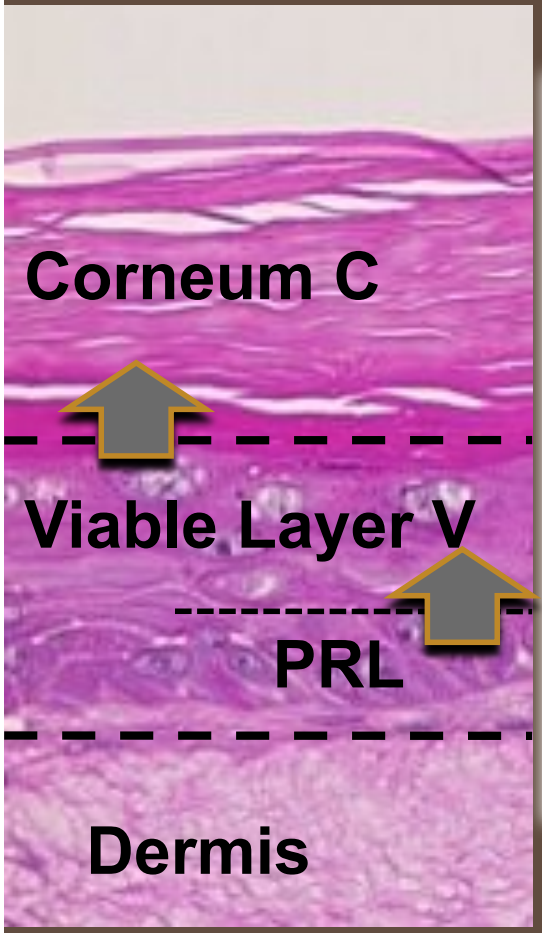
TOWARDS MODELING TONGUE EXTENSION

- Tongue extension: not only migration !!
- Interplay of proliferation, differentiation, migration during full period of epidermal remodelling
- Differention already at day 1: Keratin K1/10 staining:

PROLIFERATION ANALYSIS BY IMAGE PROCESSING



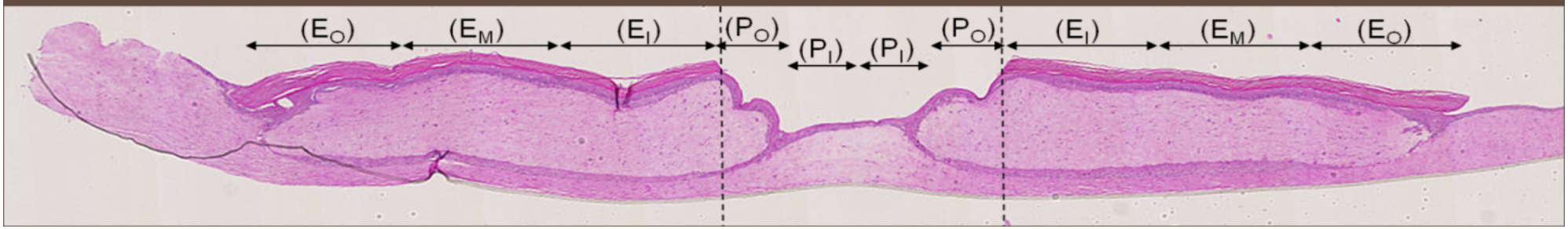
TOWARDS A MODEL OF EPIDERMAL CELL STREAMS



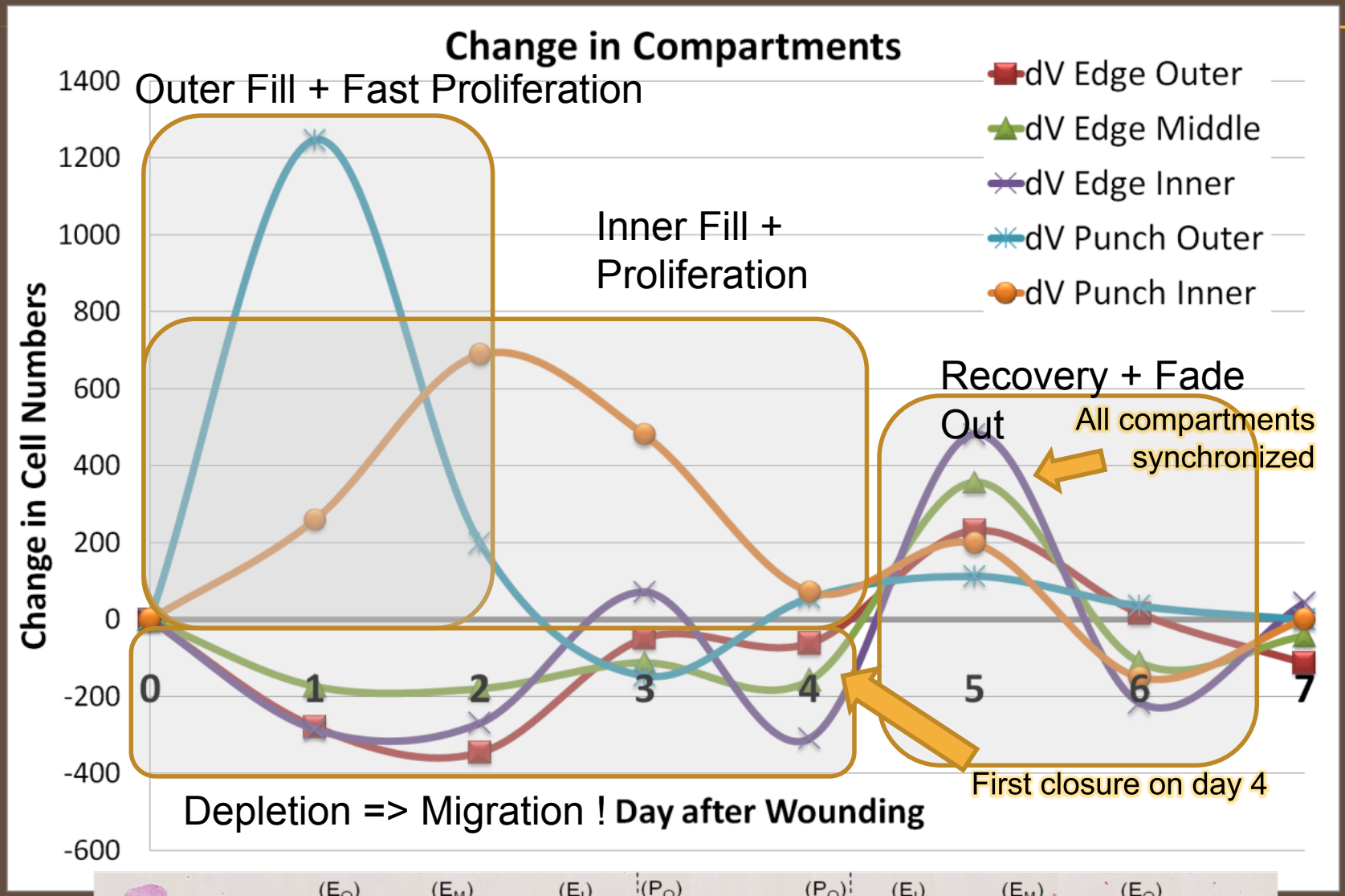
Horizontal and Vertical Cell Streams in Epidermal Wound Healing

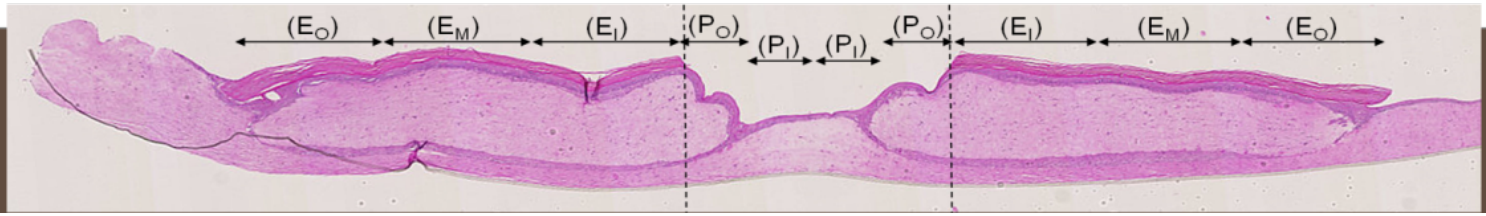
Tissue Compartment	E_O (day t)	E_M (day t)	E_I (day t)	P_O (day t)	P_I (day t)
Corneum thickness (C)	$C_{EO}(t)$	$C_{EM}(t)$	$C_{EI}(t)$	$C_{PO}(t)$	$C_{PI}(t)$
Viable compartment thickness (V)	$V_{EO}(t)$	$V_{EM}(t)$	$V_{EI}(t)$	$V_{PO}(t)$	$V_{PI}(t)$
Proliferation rate (PRL)	$PRL_{EO}(t)$	$PRL_{EM}(t)$	$PRL_{EI}(t)$	$PRL_{PO}(t)$	$PRL_{PI}(t)$

J_{EO} J_{EM} J_{EI} J_{PO}



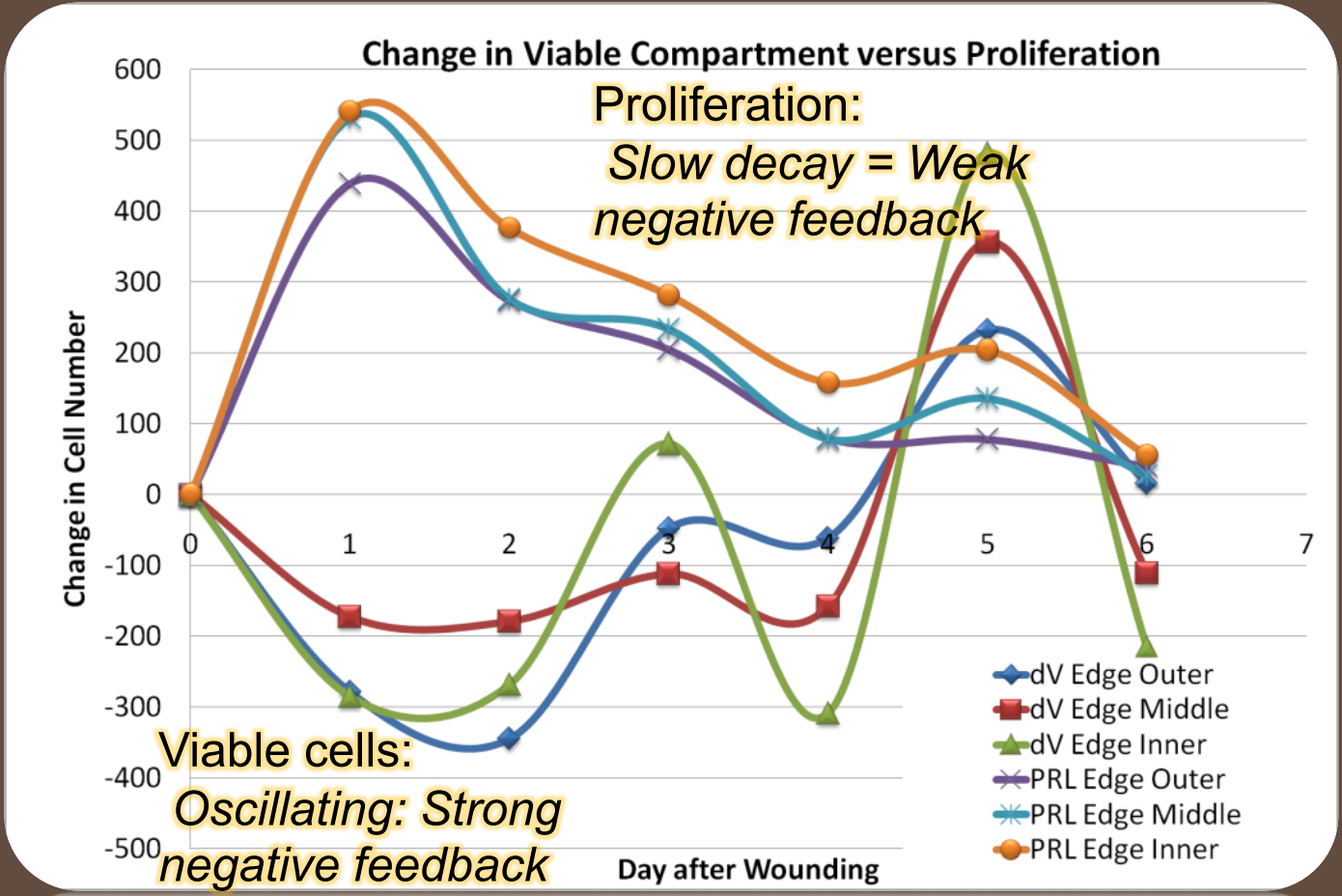
REMODELLING IN OSCILLATING WAVES





DOES PROLIFERATION INDUCE PULSING IN THE WOUND EDGE? (NO)

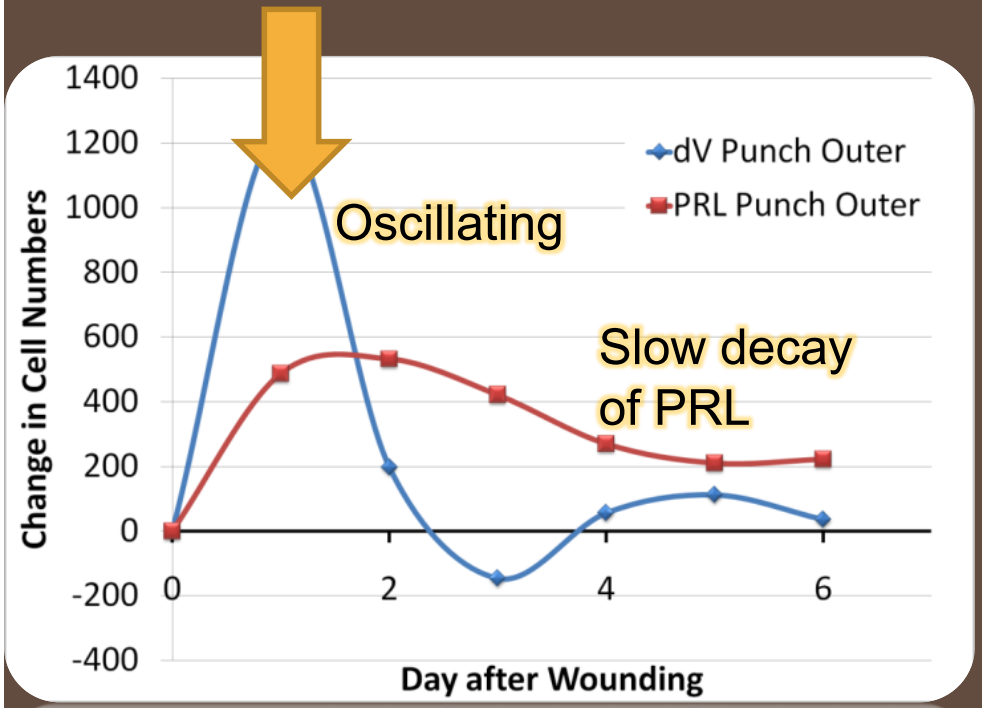
- Proliferation extending homeostasis has decay pattern => Oscillating cell efflux into wound is cause for oscillating cell numbers in viable compartment V



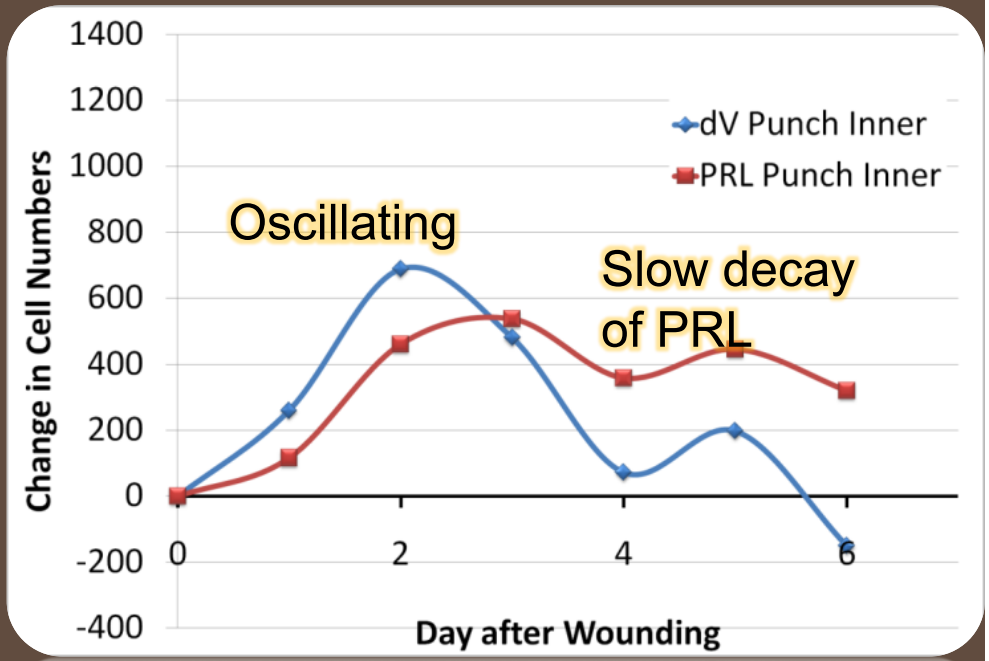
DOES PROLIFERATION INDUCE PULSING IN THE INNER WOUND? (PARTLY)

- Oscillation appears to pump some cells into the wound
- Outer wound filled by proliferation by multiplying migration
- To inner wound proliferation importance prevails

Influx into outer wound area

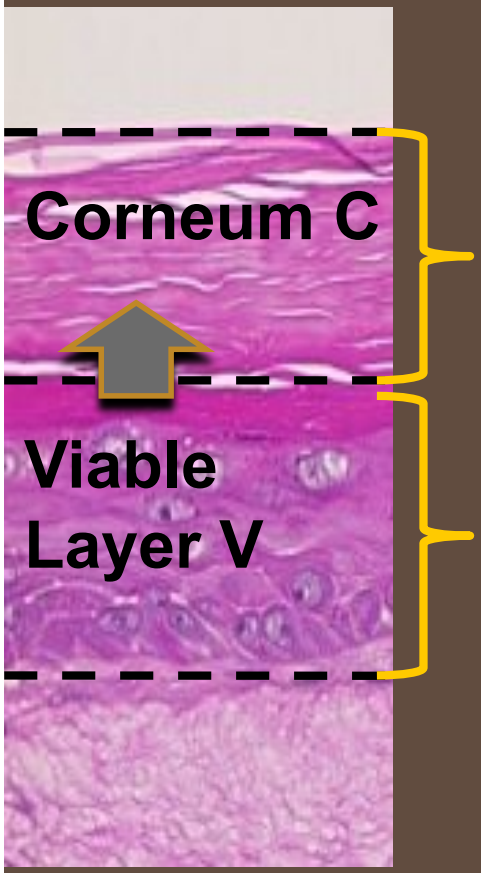


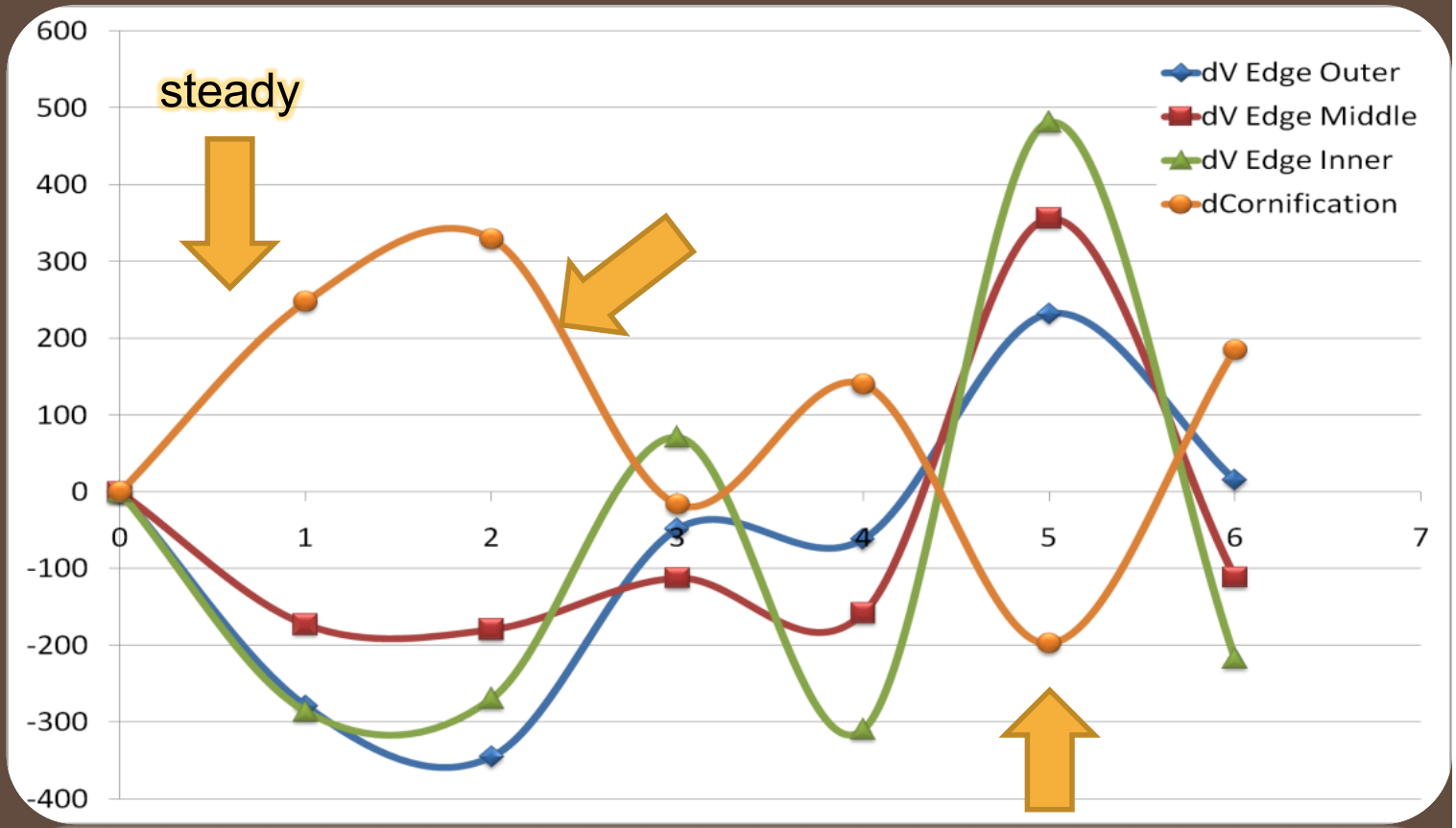
Influx into inner wound area



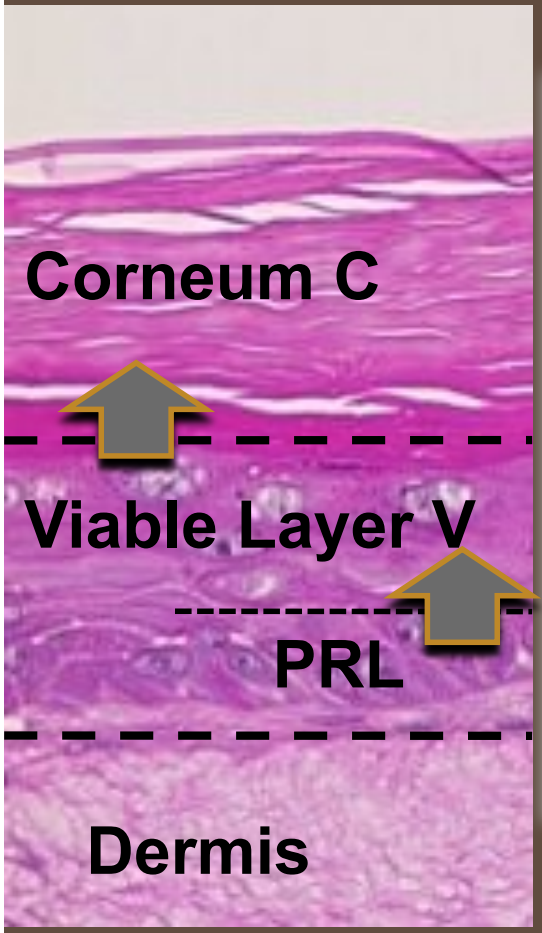
DYNAMIC DIFFERENTIATION INDUCES PULSING OF WOUND EDGE

- Differentiation measured by corneal thickness
- Initial migratory depletion triggers pulsing differentiation in wound edge





TOWARDS A MODEL OF EPIDERMAL CELL STREAMS



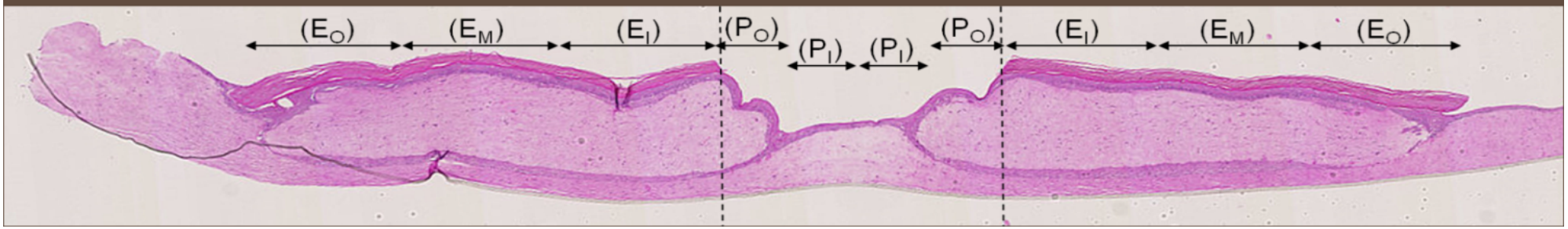
Horizontal and Vertical Cell Streams in Epidermal Wound Healing

Tissue Compartment	E_O (day t)	E_M (day t)	E_I (day t)	P_O (day t)	P_I (day t)
Corneum thickness (C)	$C_{EO}(t)$	$C_{EM}(t)$	$C_{EI}(t)$	$C_{PO}(t)$	$C_{PI}(t)$
Viable compartment thickness (V)	$V_{EO}(t)$	$V_{EM}(t)$	$V_{EI}(t)$	$V_{PO}(t)$	$V_{PI}(t)$
Proliferation rate (PRL)	$PRL_{EO}(t)$	$PRL_{EM}(t)$	$PRL_{EI}(t)$	$PRL_{PO}(t)$	$PRL_{PI}(t)$

Pulsing differentiation over time (negative feedback)

Weakly pulsing migration pump over time

Strong upregulation & moderate negative feedback

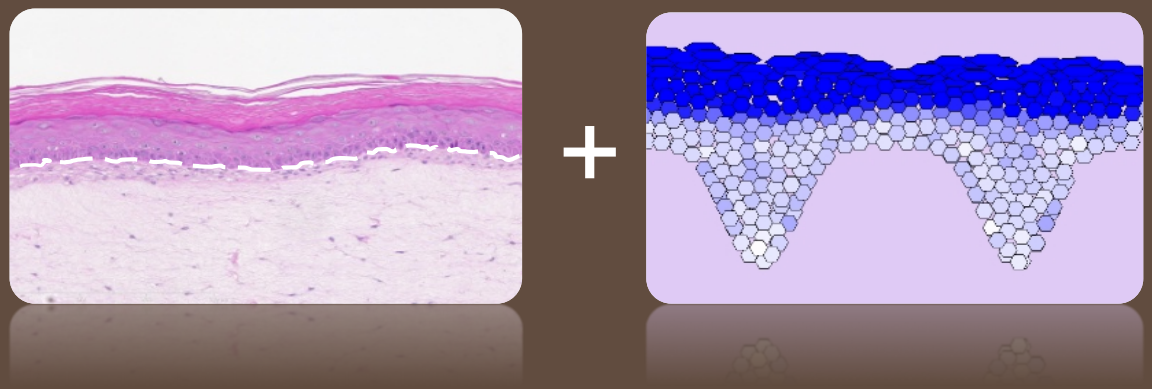


TOWARDS QUANTITATIVE SYSTEMS BIOLOGICAL TISSUE MODELS BY USING WHOLE SLIDE IMAGING

1. Systems biology drives towards tissue models
2. Quantitative spatial of tissues are missing: why whole slide imaging is essential for systems biology of tissues
3. PART A: Generating data : Quantifying spatial protein expression data in human skin (Full slides & TMAs)
    1. Application of quantitative spatial profiles for
        1. Reconstructing networks
        2. Building tissue classifiers
        3. Qualitative network reasoning
4. PART B: Generating data: Quantitative morphological analysis of dynamic wound healing (Full slides)
    1. Analysis of cellular streams in tissues

KEY CONCEPT

- Systematic pertubation studies of tissue require integration of
  - in vitro-models (organotypic cell culture systems)
  - in silico-models
- Realisation
  - Quantitative data from native tissue
  - Quantitative data from in-vitro cultures using Whole-Slide Imaging
  - Multiple technologies (multipPhoton, confocal) will complement
  - Computational multi-cellular multi-agent platform for this data



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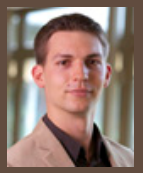
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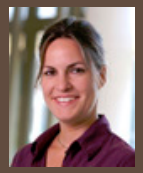
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