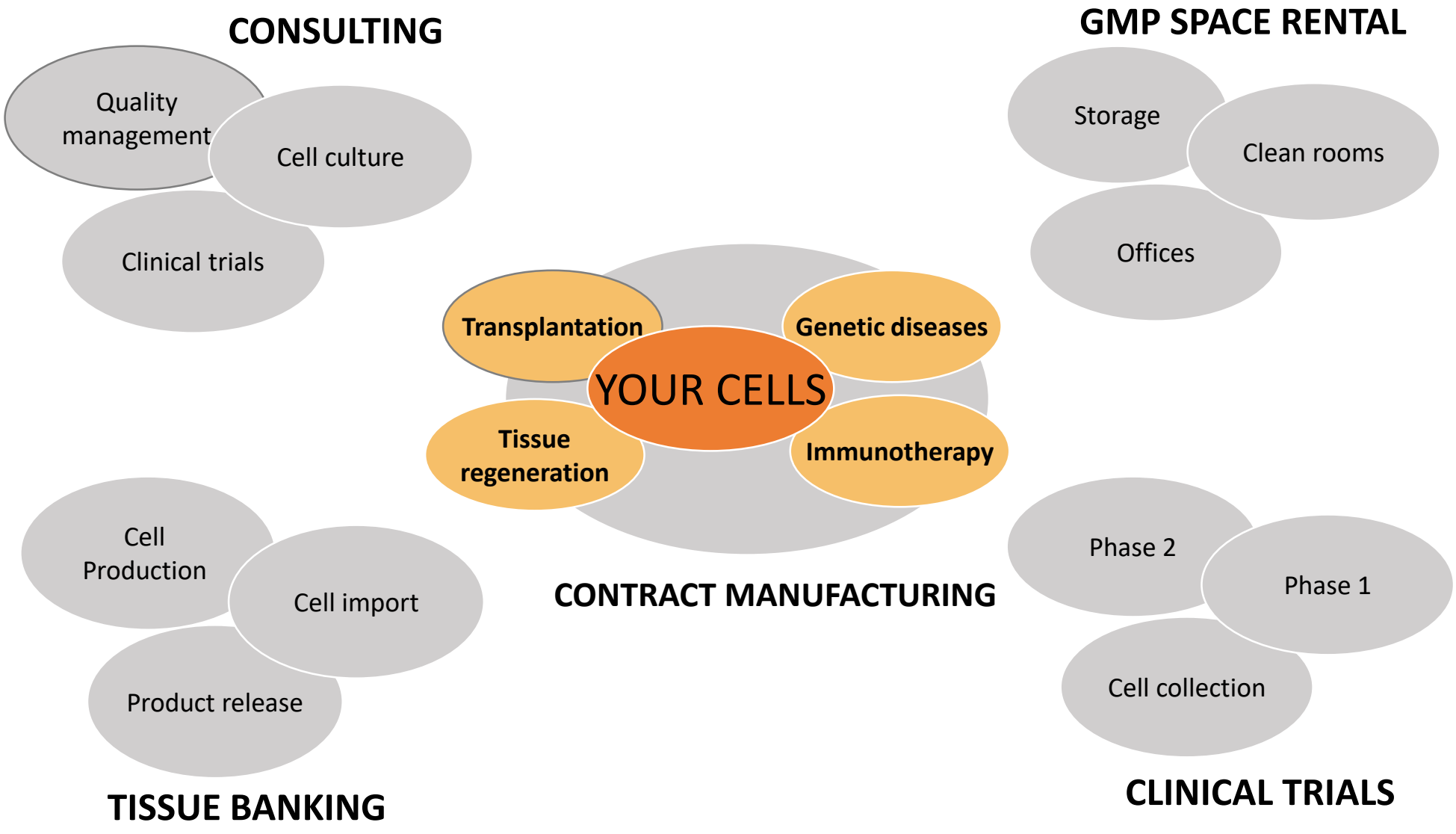
Laboratory of Cell and Gene Therapy (LTCG)

From R&D to Contract Manufacturing for clinical trials

CHU de Liège

6 scientists, 6 technicians
Pr Yves BEGUIN


CONSULTING
Quality management
Cell culture
Clinical trials
GMP SPACE RENTAL
Storage
Clean rooms
Offices
Transplantation
Genetic diseases
YOUR CELLS
Tissue regeneration
Immunotherapy
CONTRACT MANUFACTURING
Cell Production
Cell import
Product release
TISSUE BANKING
Phase 2
Phase 1
Cell collection
CLINICAL TRIALS

Service proposal & technical specificities

- A **GMP-approved facility** offering
  - ❑ Cell collection (from patients and healthy volunteers)
  - ❑ Cell selection & expansion
  - ❑ Stem cell processing & infusion
  - ❑ Manufacturing of clinical-grade cell and/or tissue engineering products
  - ❑ Cryopreservation & storage
  - ❑ Validated product shipping procedures
  - ❑ Consulting (quality management, clinical trials, cell culture)

- **Distinctive specificities:**
  - 3 **FAMHP-approved tissue banks**:
    - Hematopoietic stem cells
    - Cord blood (> 3.500 validated units)
    - Non-hematopoietic cells
  - **R&D unit** to transfer innovative cell technologies to the clinic
  - **ATMP production lab**
  - **GMP space rental**

Contact

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Manufacturing:
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+32 4 242 77 60

a powerful platform for screening beta cell therapeutics

K. Hellemans

Diabetes Research Center – VUB

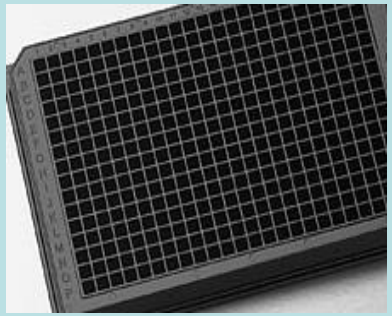
OPUS NV

BetaSCREEN, our mission

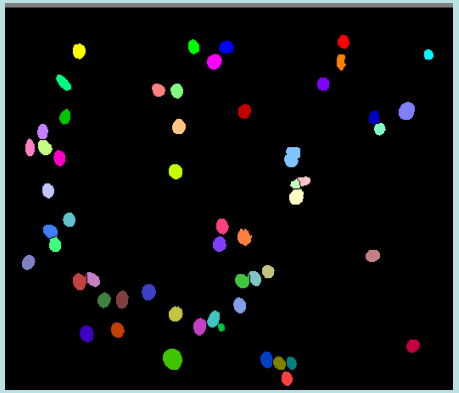
Need for Drugs that Enhance Functional Beta Cell Mass

Currently used drugs that stimulate insulin release were identified through their acute effects on the secretory function of isolated pancreas or of islet preparations. **The search for drugs that enhance functional beta cell mass requires assays in which the number of beta cells and their insulin producing capacity are monitored in parallel over several days**.

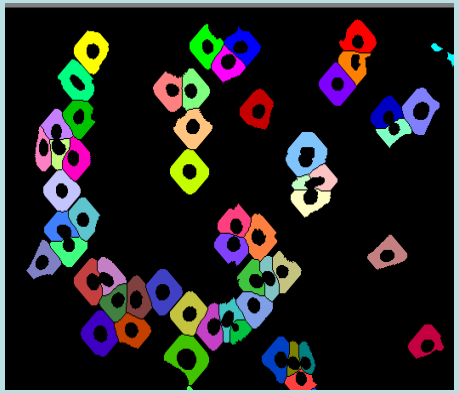
Primary beta cells of rodent, pig or human origin, or hu-iPS derived beta cells are FACS sorted to purities of > 95% insulin positive cells



Beta cells are seeded in 96w or 384w plates in appropriate culture media and cultured up to three weeks



Whole well Images are captured using a BD Pathway imager for the analysis of cell viability, total cell numbers, proliferation and expression of specific markers.



The images are segmented and divided into regions of interest where desired, measurement such as fluorescence intensity and distribution of specific markers and morphological features are made.

Automation at Single Cell Scale

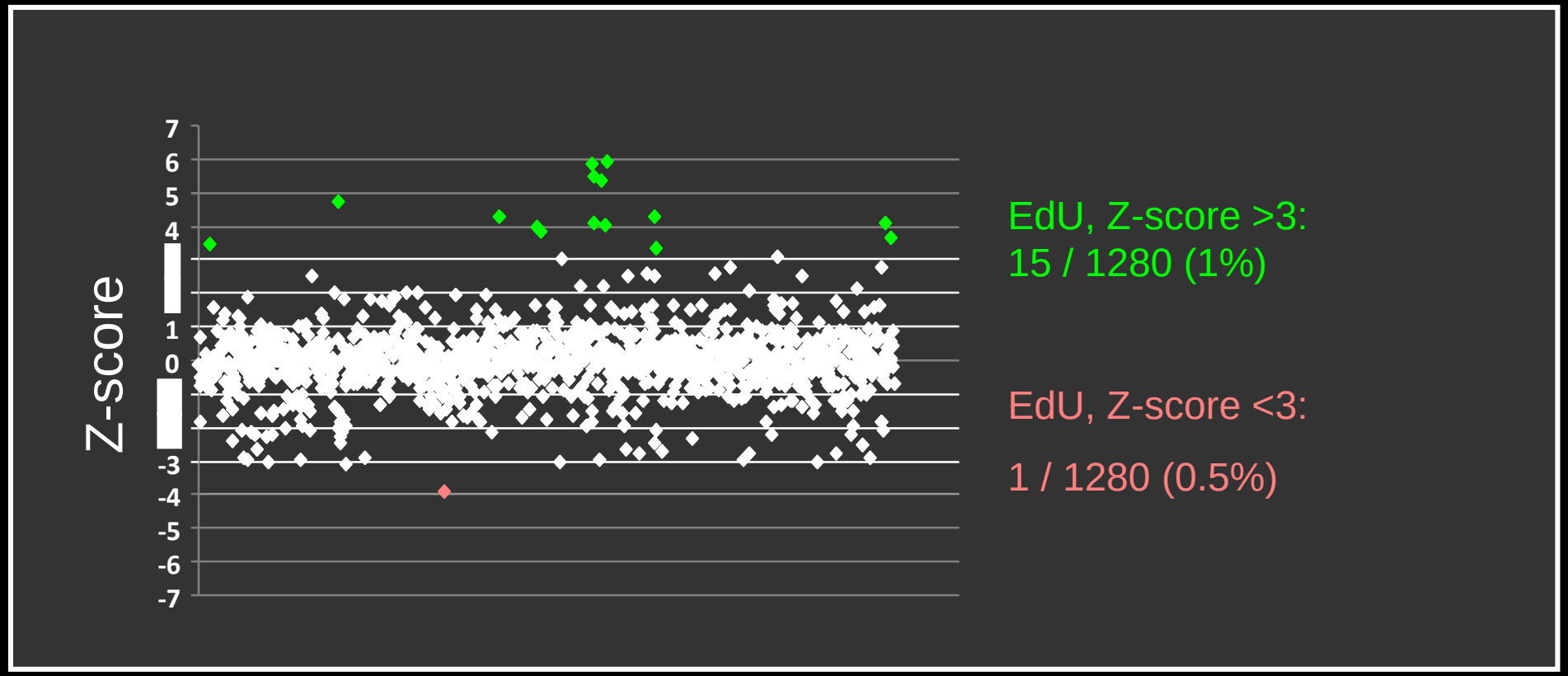
A unique functional cytometry platform developed by the Diabetes Research Center - VUB

Adapted to the study of chemical entities with positive or negative, acute or chronic effects on beta cells of primary or stem cell origin

Screening Achievements

1280 compounds 1µM,

Read out: stimulatie van proliferatie

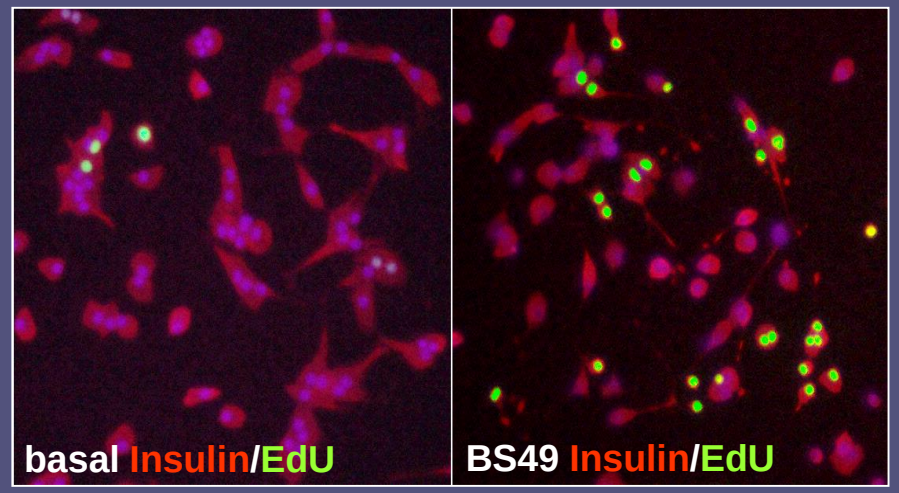


Screening Achievements

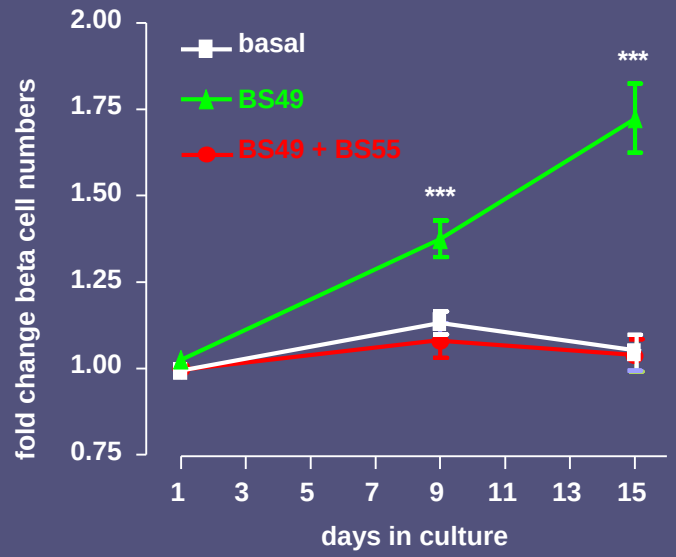
A class of small molecule compounds (represented by BS49) that reproducibly shifts beta cells into proliferative activity has been identified together with their intracellular target

Dose-response curves are used to compare stimulating compounds and/or to detect compounds that suppress/inhibit stimulated beta cell proliferation

Our **platform is ready to use for compound screening** and the selection of compounds that sustain or increase the functional beta cell mass in diabetic patients through preservation of beta cell survival and/or activation of their (re)generation



Beta cells in basal medium (left panel) exhibit a small percentage of cells in proliferative activity (EdU-positive, green). When the small molecule BS49 is added to the basal medium, more than 30 % of the beta cells become EdU-positive (right panel).



Increase of living beta cell numbers over 15 days in basal medium and in the presence of a stimulatory molecule (BC-49) and/or a inhibitory molecule (BC-55). (mean ± SDEV, N=5)

These observations are indicative for a therapeutic potential that needs to be further explored in collaboration with an industrial partner

BetaSCREEN unique features

Validated screening platform in which

Beta cells are cultured using serum-free media allowing:

... Parallel assessment of viability, cell number, proliferation and insulin production

... Detection of short and long-term effects on each read-out

… Agents have been identified with positive (and negative) effects on beta cell survival and number and are used as positive controls

... Models have been developped to extrapolate in vitro predictions to their in vivo relevance

MULTI LEVEL SCREENING PLATFORM

BetaSCREEN: HIT identification

LEVEL 1 – 6 days	Acute effects on viability, toxicity, insulin production
LEVEL 2 – 15 days	Chronic effects on beta cell number, proliferation & phenotype

BetaPLUS: From HIT to LEAD

LEVEL 3 – PRECLINICAL MODELS selected conditions	Effects on functional beta cell mass (pancreas / beta cell graft)
LEVEL 4 – Clinical trials selected conditions – specific questions	Clinical Trial Unit – Uzbrussel In house expertise with phase I and phase II trials to move to a next level in your discovery pipeline ....

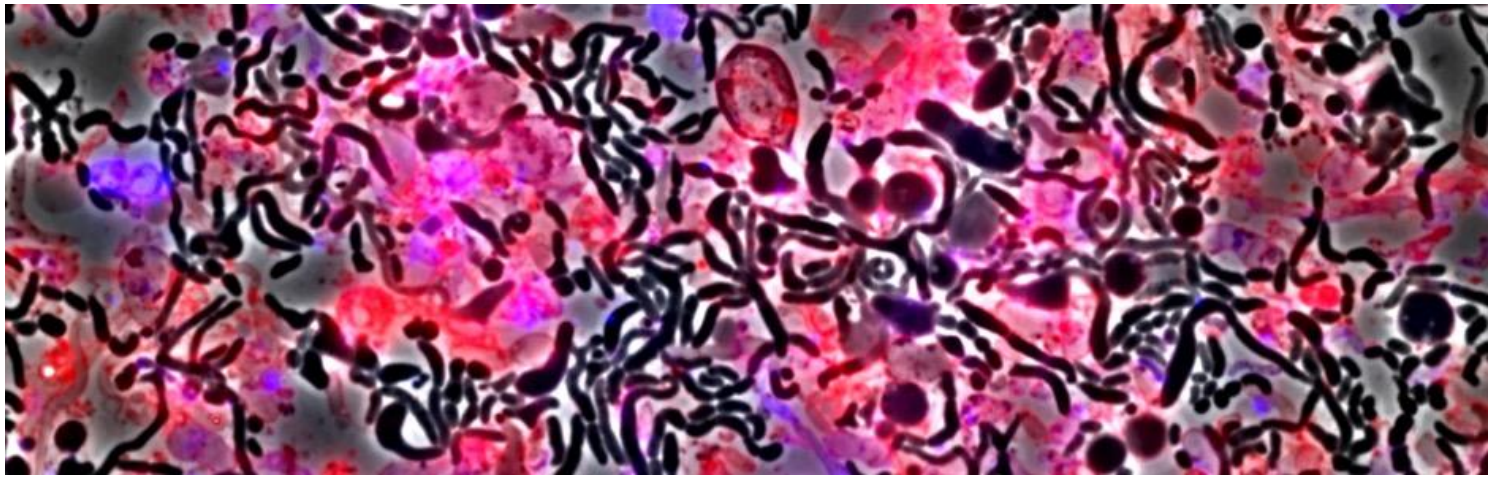
Contact: Karine.Hellemans@vub.be

Website: betacelltherapy.com

Further reading and more information:

- Assefa Z, et al., *PlosOne* 2014;9(1):e85174.
- Assefa Z, et al., *Am J Physiol Endocrinol Metab. 2016: 2016;311(4):E698-E705*
- *(*Akbib S, et al., *PLoSOne. 2019;14(2):e0212210*.

Collet lab
Industrial valorization of fundamental research



BiR&D
19/03/2019

UCLouvain

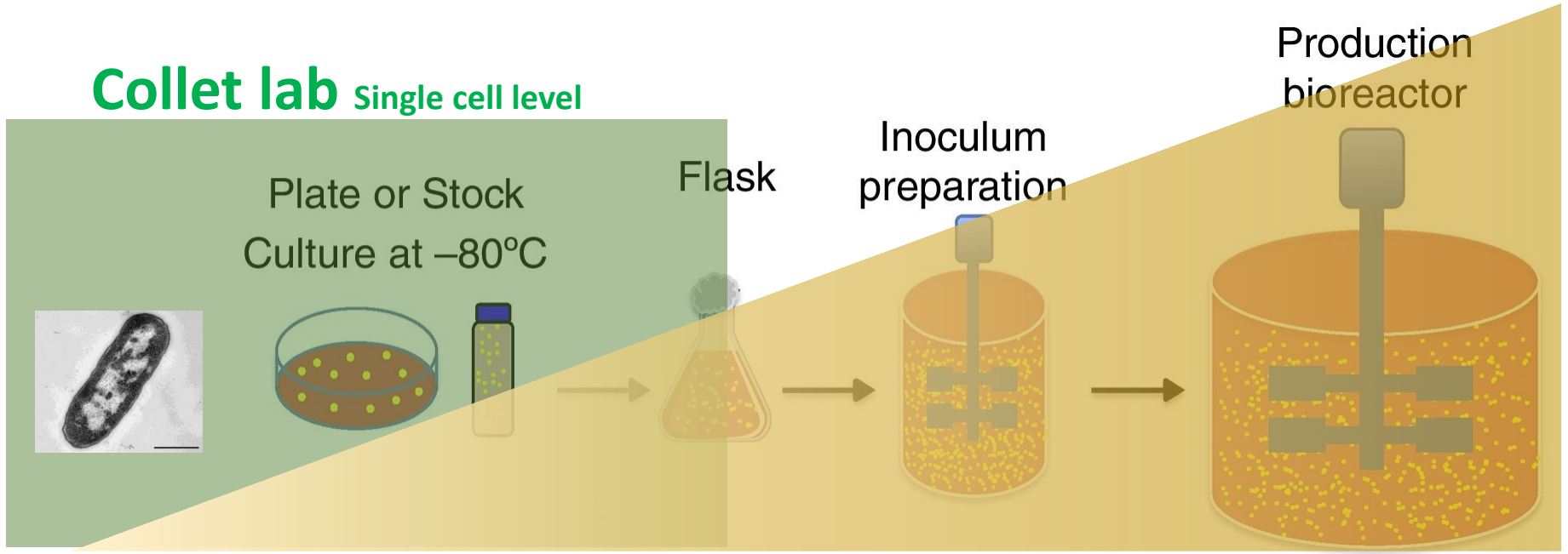
BiR&D

de Duve INSTITUTE

Collet Microbial Factory

Industrial production with bacteria can be divided into 2 main stages:

1. UPS = Upstream process → scale up from the inoculum to the bioreactor
2. DSP = Downstream process → from cell harvesting to final product

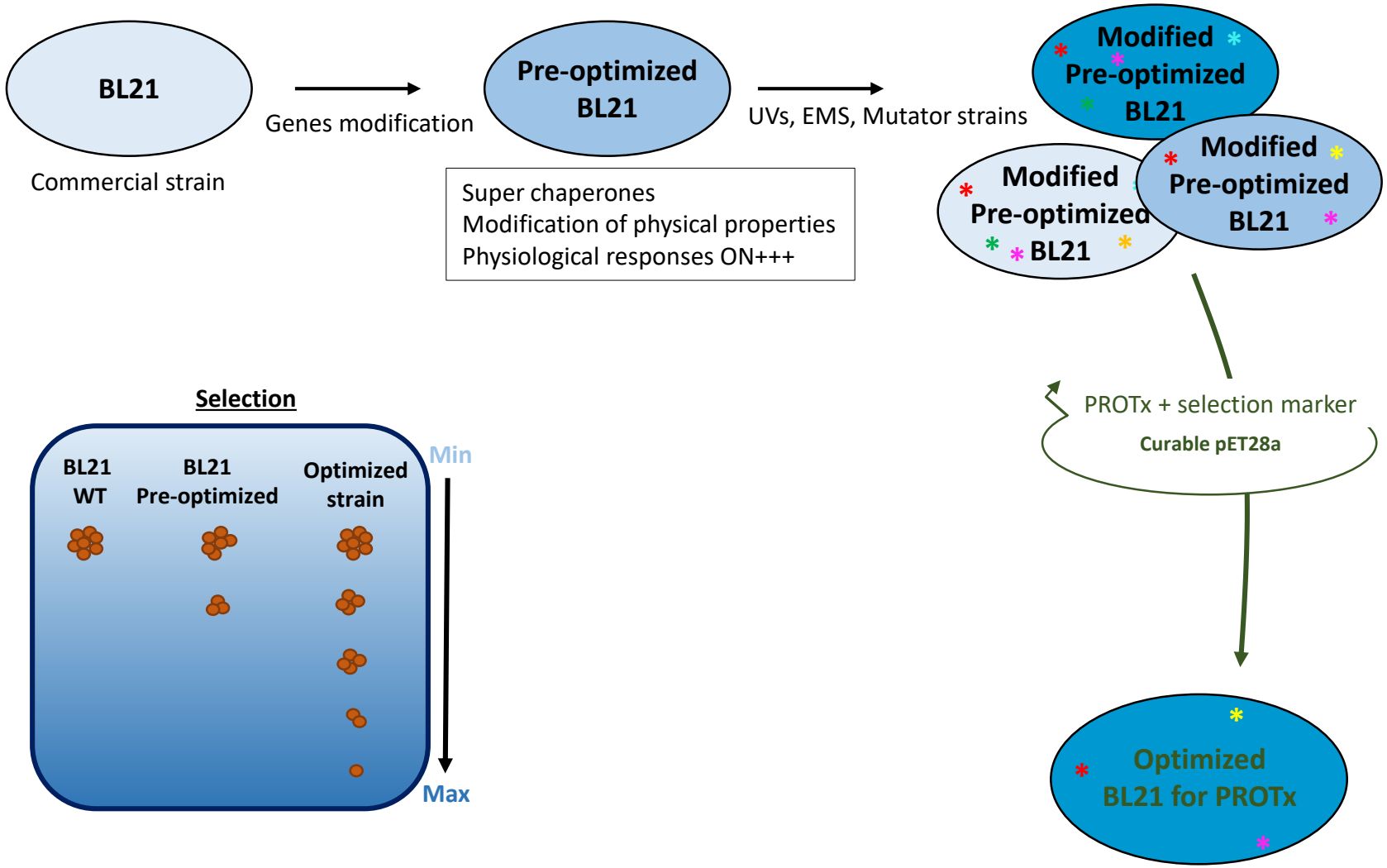


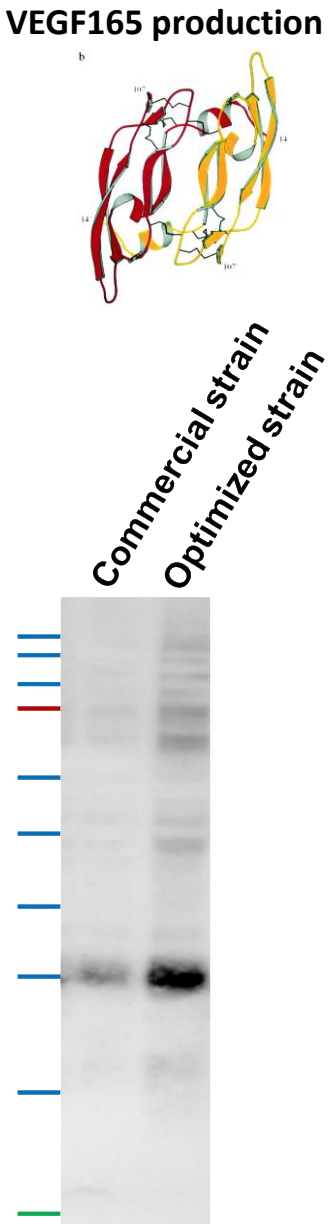
DSP

Impact of the improvement increases with the scale up

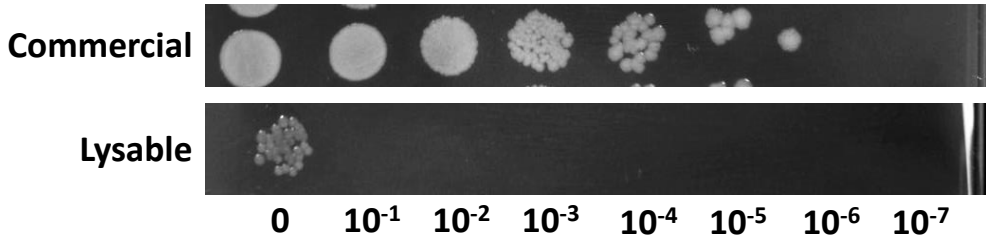
Improving protein production for the industry

Periplasmic protein production

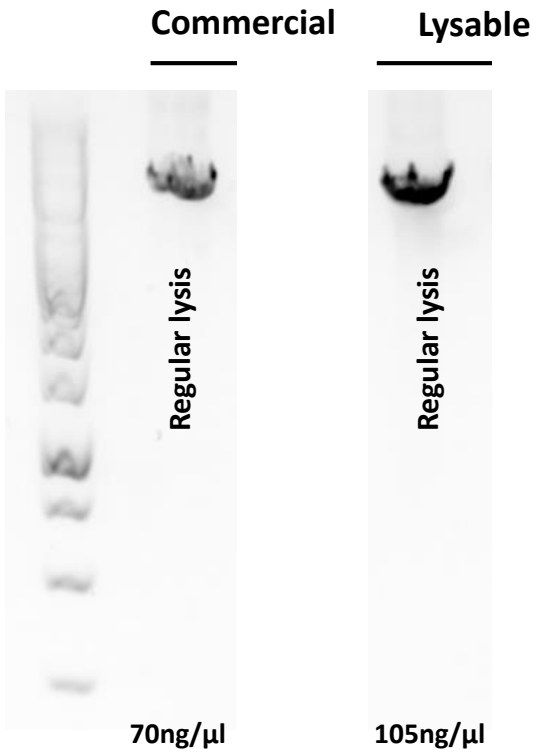


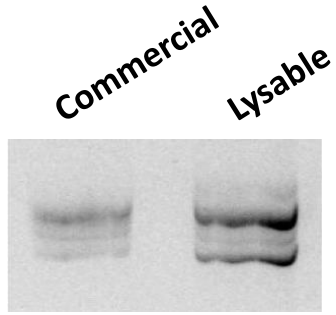


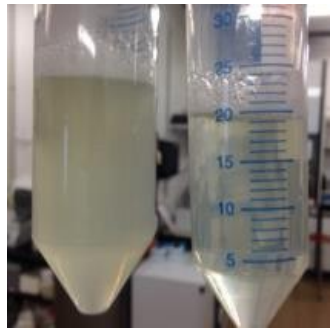
Increasing strain lysability for plasmidic DNA production



Lysable Lyses 100.000 times more than the commercial







The lysate for plasmid preparation is **clearer** and **less viscous** in Lysable strain

The volume of cell debris after Neutralization is more compact → release **more plasmidic fraction** and **facilitates the DSP**

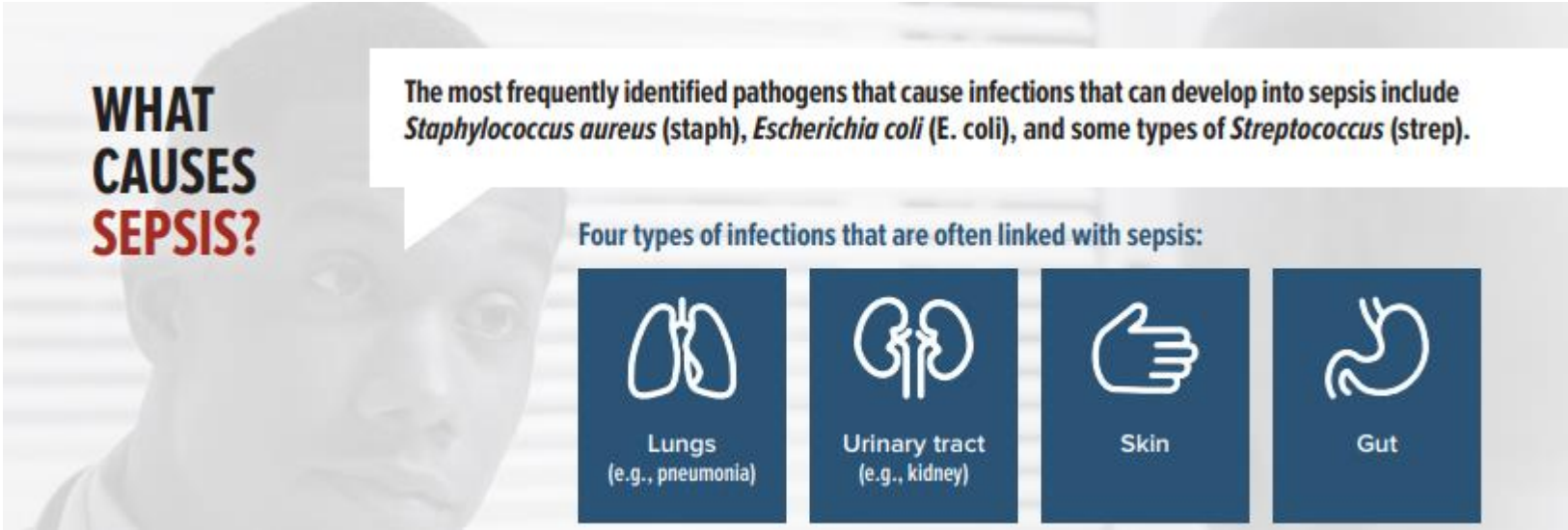
KU LEUVEN

A therapy for sepsis

Dr. Bart Landuyt
Functional Genomics & Proteomics

Sepsis: a global health problem

- Every year: 35 million people
- 30 % mortality rate
- 3rd greatest cause of in-hospital mortality
- Overtakes myocardial infarction and stroke
- Incidence still increasing
- WHO priority



KU LEUVEN

LPS tolerance mouse model for sepsis

High dose LPS

Mouse dies

Low dose LPS

Mouse survives and becomes tolerant

Low dose LPS +High dose LPS

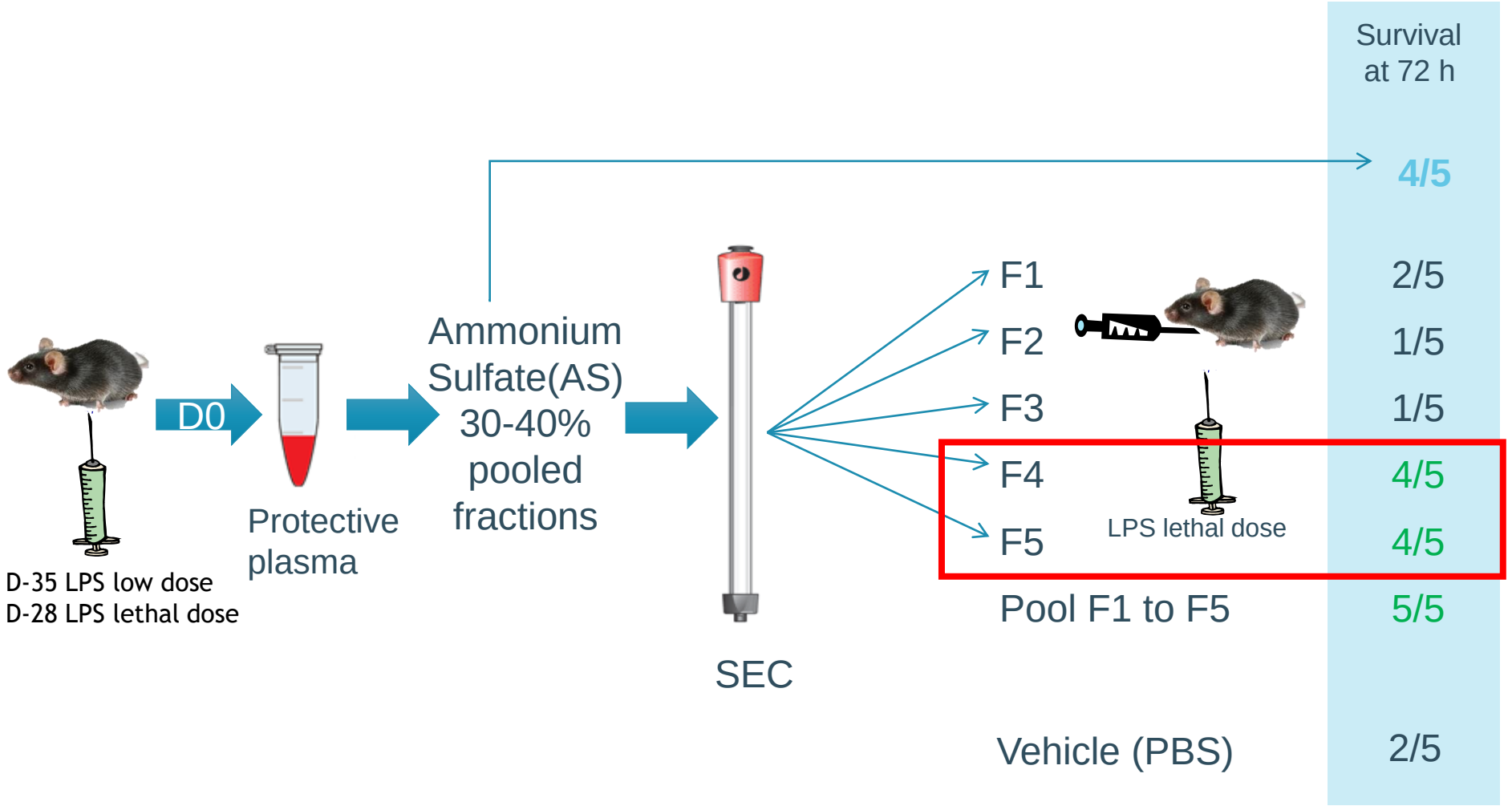
Tolerant mouse survives lethal dose LPS

+ High dose LPS

Protection is transferrable through plasma

- Still there after 35 days
- Protective factor active after LPS administration
- Heat resistant
- B cells are necessary for production

Proteomics workflow



Results: 4-6 proteins for therapeutic development!

Protein ID	Upregulated in 2D DIGE	Upregulated in TMT	Downregulated in B-cell deficient mice	Upregulated at # time points	Upregulated in sepsis survivors/ downregulated in sepsis non-survivors
Protein A	✓	✓	✓	✓	✓
Protein B	✓	✓	✓	✓	✓
Protein E	✓	✓	✓	✓	✓
Protein K	NA	✓	✓	✓	✓
Protein J	NA	✓	✓	✓	NA
Protein F	NA	✓	✓	✓	✗
Protein H	✓	✓	✓	✗	✗
Protein M	NA	✓	NA	NA	NA
Protein C	✓	✓	✓✗	✗	✓
Protein D	✓	✓	✓✗	✗	✗
Protein I	NA	✓	✗	✗	✓
Protein G	✓	✓	✗	✓	✗
Protein L	NA	✓	✗	✗	✗

Contact

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Functional Genomics & Proteomics

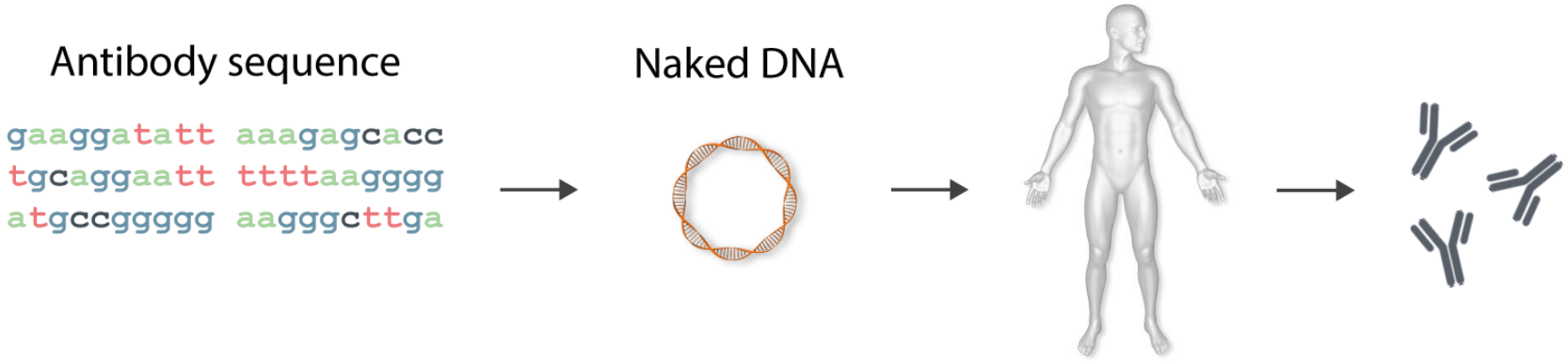
Naamsestraat 59

3000 Leuven

KU LEUVEN

DNA-based Antibody Therapeutics

the patient as medicine producer



Patients receive the antibody DNA, rather than the protein, allowing the body to produce the antibody of interest

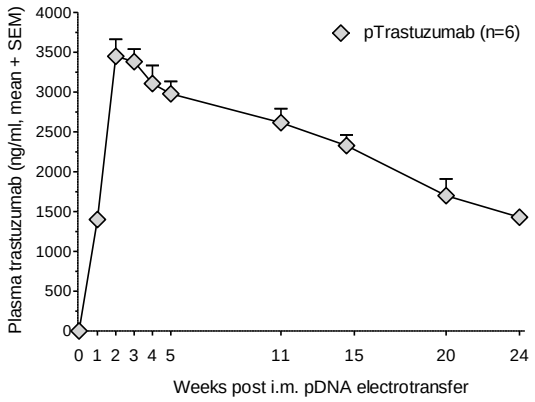
- LOWER development time & cost (DNA versus protein COG)
- REDUCED administration frequency (prolonged *in vivo* production)
- INCREASED efficacy (combination therapies or local application)

PharmAbs
The KU Leuven Antibody Center

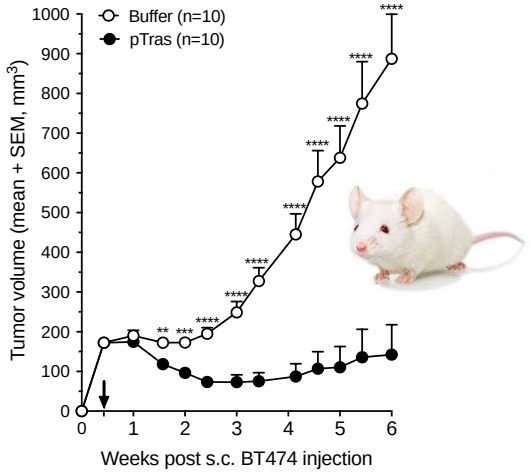
Intramuscular DNA delivery leads to prolonged functional mAb expression

INTRAMUSCULAR DNA-BASED ANTIBODY THERAPY

Prolonged mAb expression following single intramusclar DNA-based mAb electrotransfer (dose-response, repeated dosing feasible)

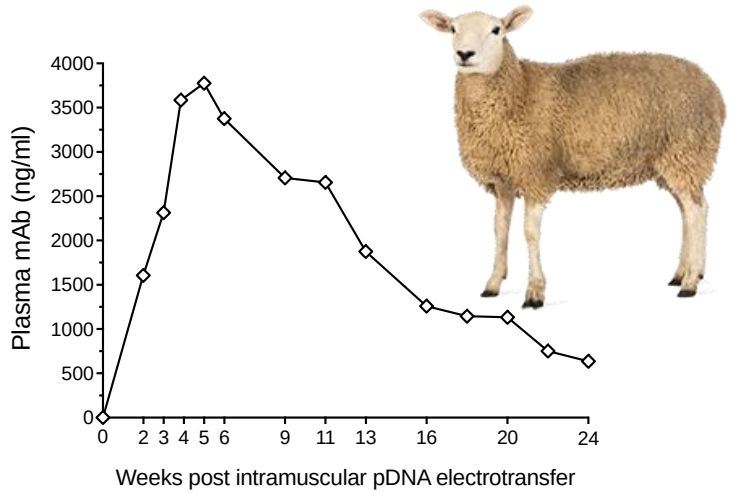


Therapeutic efficacy in a breast cancer model



PROOF OF CONCEPT IN MICE (*Hollevoet et al. Oncotarget, 2018)*

SUCCESSFUL TRANSLATION FROM MICE TO SHEEP



Using clinical-grade electroporation device, therapeutically relevant plasma mAb concentrations are obtained in 40-70 kg sheep (dose-response, repeated dosing feasible)

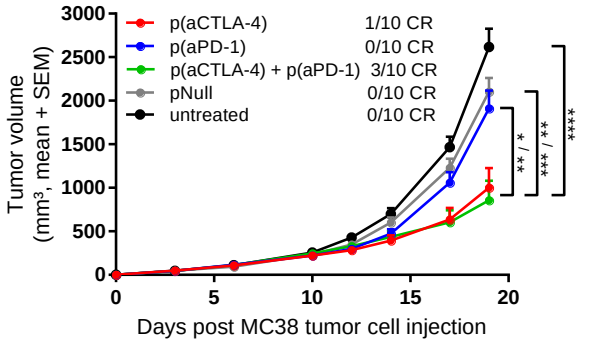
CLOSING THE GAP TO THE CLINIC

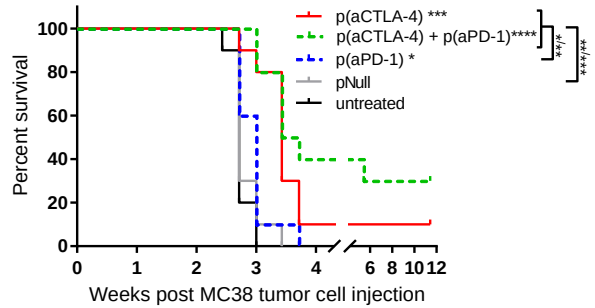
PharmAbs
The KU Leuven Antibody Center

Intratumoral DNA delivery is an effective and safe immunotherapy approach

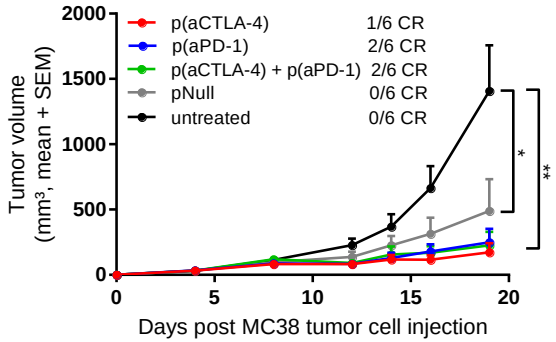
DNA-BASED IO ANTIBODY THERAPY

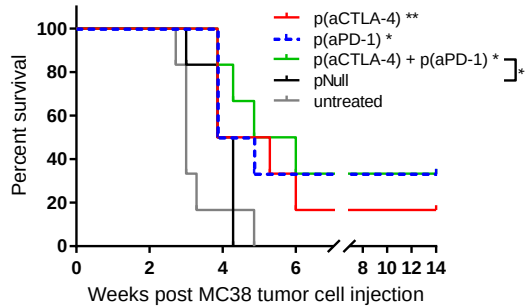
Intramuscular electrotransfer





Intratumoral electrotransfer





INTRAMUSCULAR AND INTRATUMORAL DELIVERY SHARE EFFICACY

Our data support potential benefits of intratumoral DNA-based delivery of immunomodulatory mAbs

- local efficacy and long-term anti-tumor immunity
- platform for DNA-based combinations

efficacy

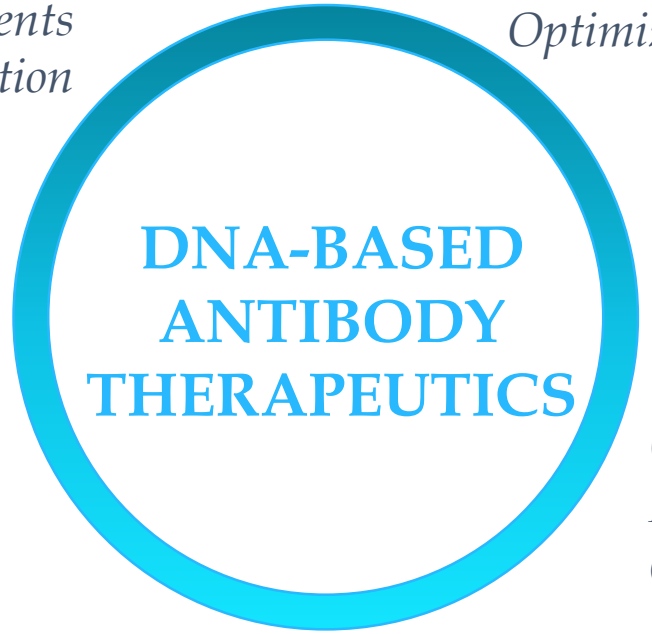
- limited systemic mAb exposure
- transient mAb expression

biosafety

PAVING THE WAY FOR LOCAL DNA-BASED IMMUNOTHERAPY

PharmAbs
The KU Leuven Antibody Center

R&D focus areas & collaboration opportunities



ANTIBODY TECH

Full-length and fragments
Sequence optimization

PLASMID DNA

Optimized cassettes & backbones

TARGET TISSUES

Muscle & tumor

TARGET DISEASES

(Immuno) oncology
Infectious diseases
CNS

CLINICAL TRANSLATION

Clinical electroporation devices
Large animal models

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PharmAbs
The KU Leuven Antibody Center

EGAMI

Presented by Wouter Van Putte, PhD – 2019

Universiteit Antwerpen

EGAMI is a **multi-disciplinary biomedical imaging platform** for **pre-clinical and clinical research** (especially neurology) within **Antwerp University** and **Antwerp University Hospital (5 research groups)**

… covering a myriad of imaging techniques:

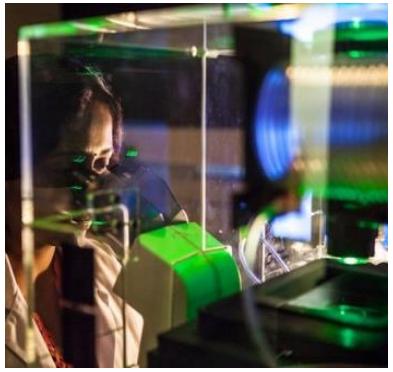
- **MRI**
- **PET/SPECT**
- **CT**
- **Electron and Fluorescence microscopy**

… including **advanced image processing (CT, MRI, High Content Analysis)**

EGAMI*image*
University of Antwerp

5 multi-disciplinary research groups

Advanced Microscopy



Laboratory for Cell Biology and Histology (**CBH**)

Small Animal In vivo Imaging

Bio-imaging Lab (**BIL**)

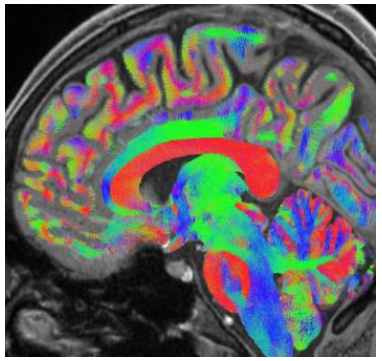
Molecular Imaging lab – pre-clinical (**MICA**)

Medical Imaging in Patients

University Hospital Antwerp (**UZA**): **Nuclear medicine Radiology**

Molecular Imaging (**MICA**)

Image Processing



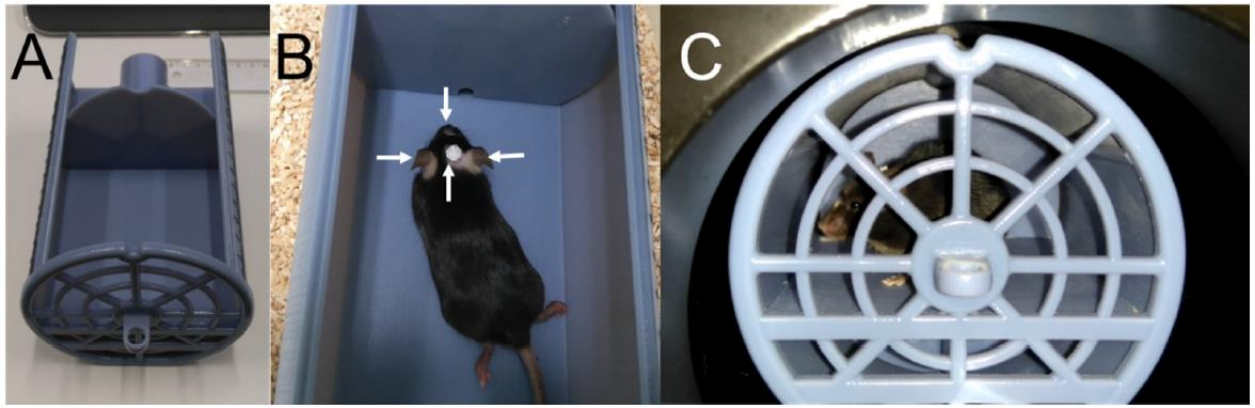
VISION LAB

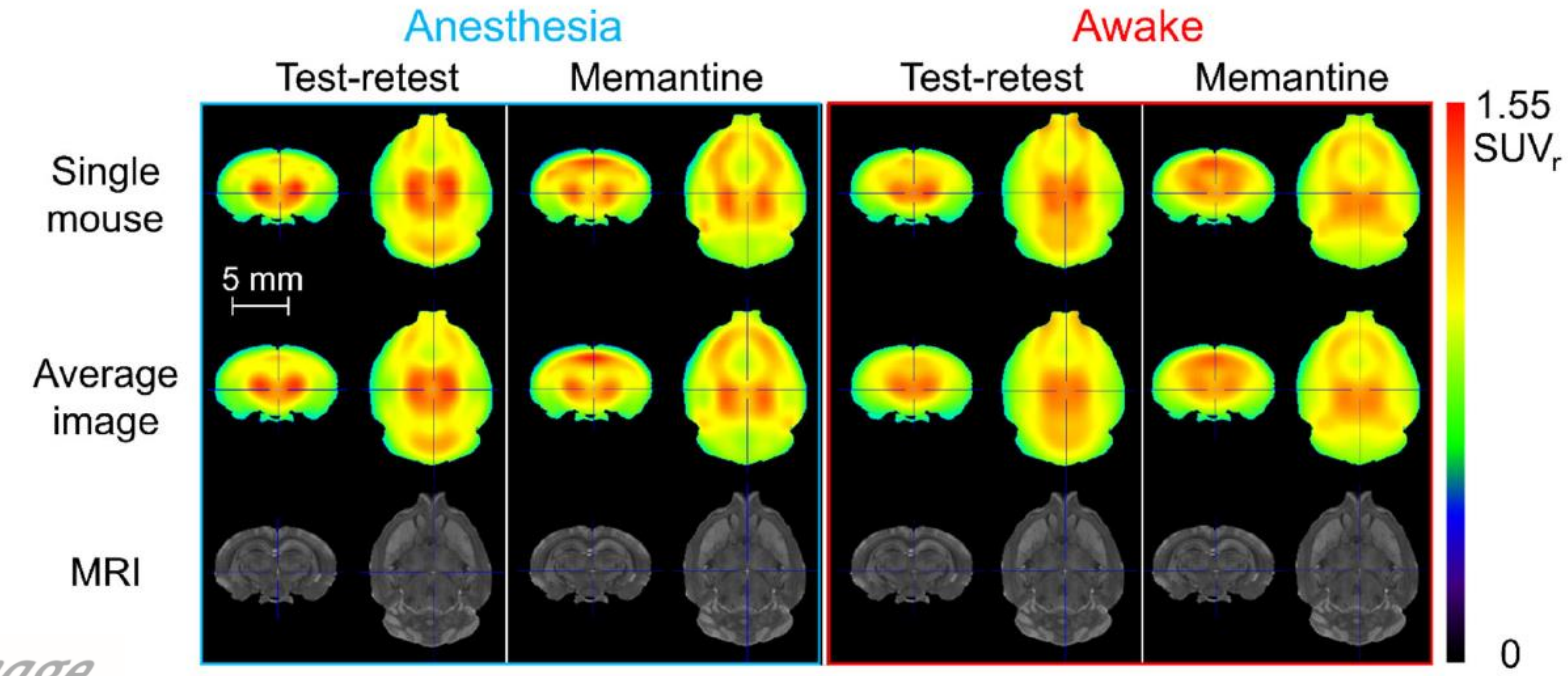
CBH

MICA

Case I: Awake PET imaging

MICA



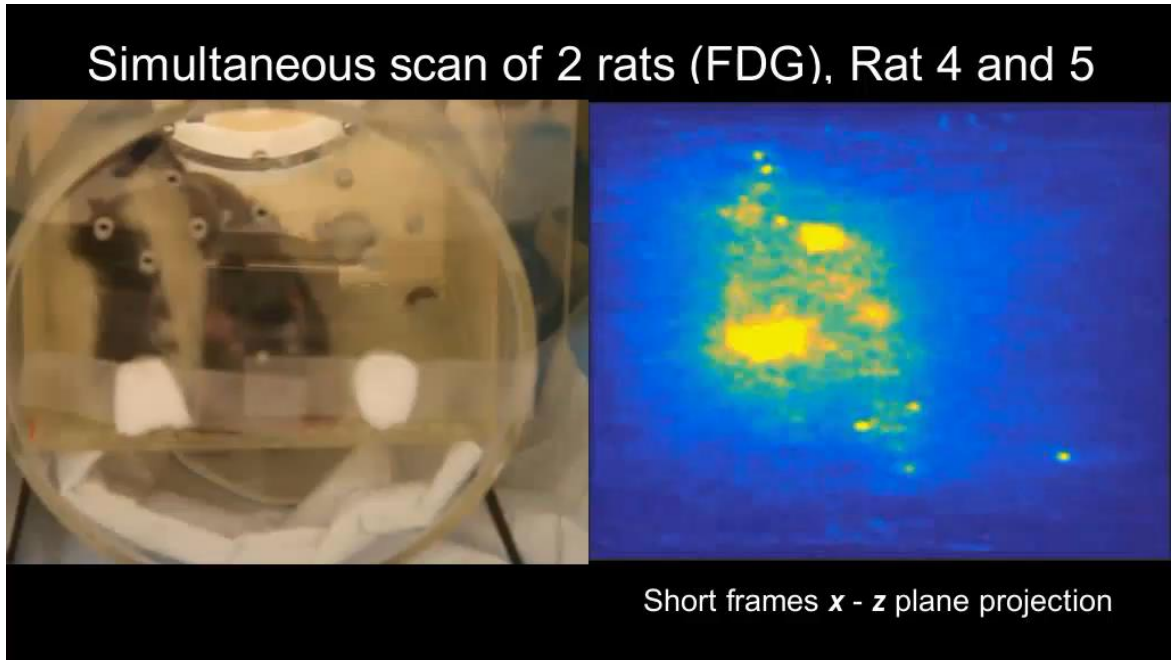


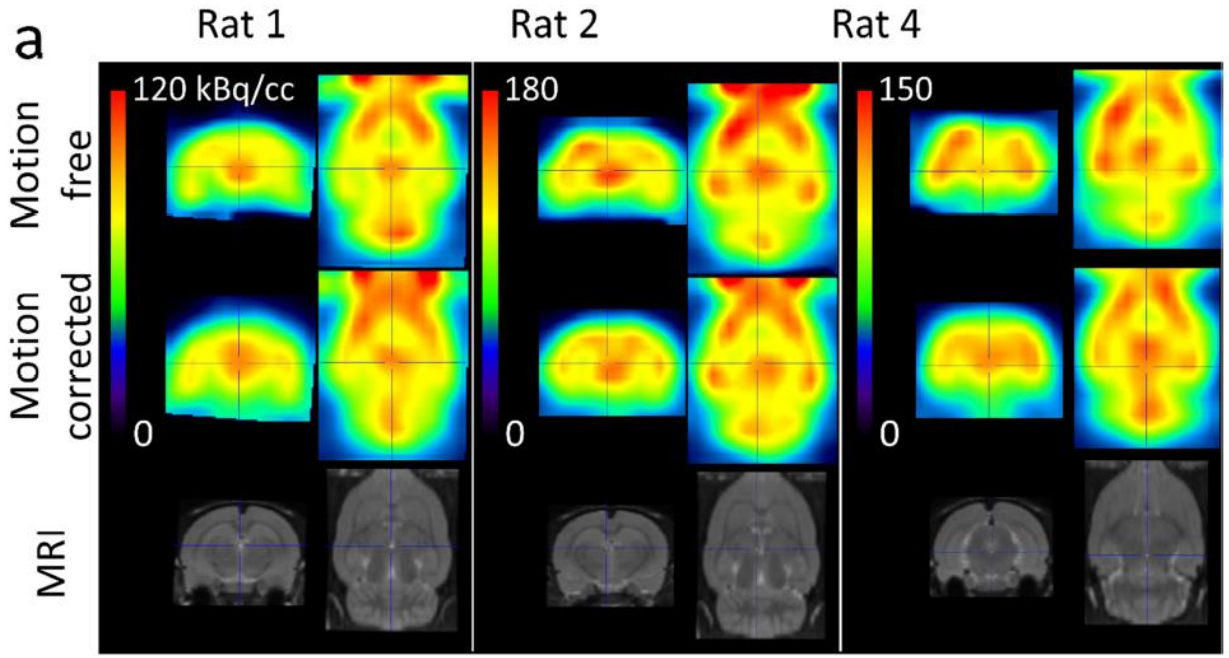
EGAMI image
University of Antwerp

Miranda et al. J Nucl Med. 2018.

Case I: Awake PET imaging of interacting animals

MICA



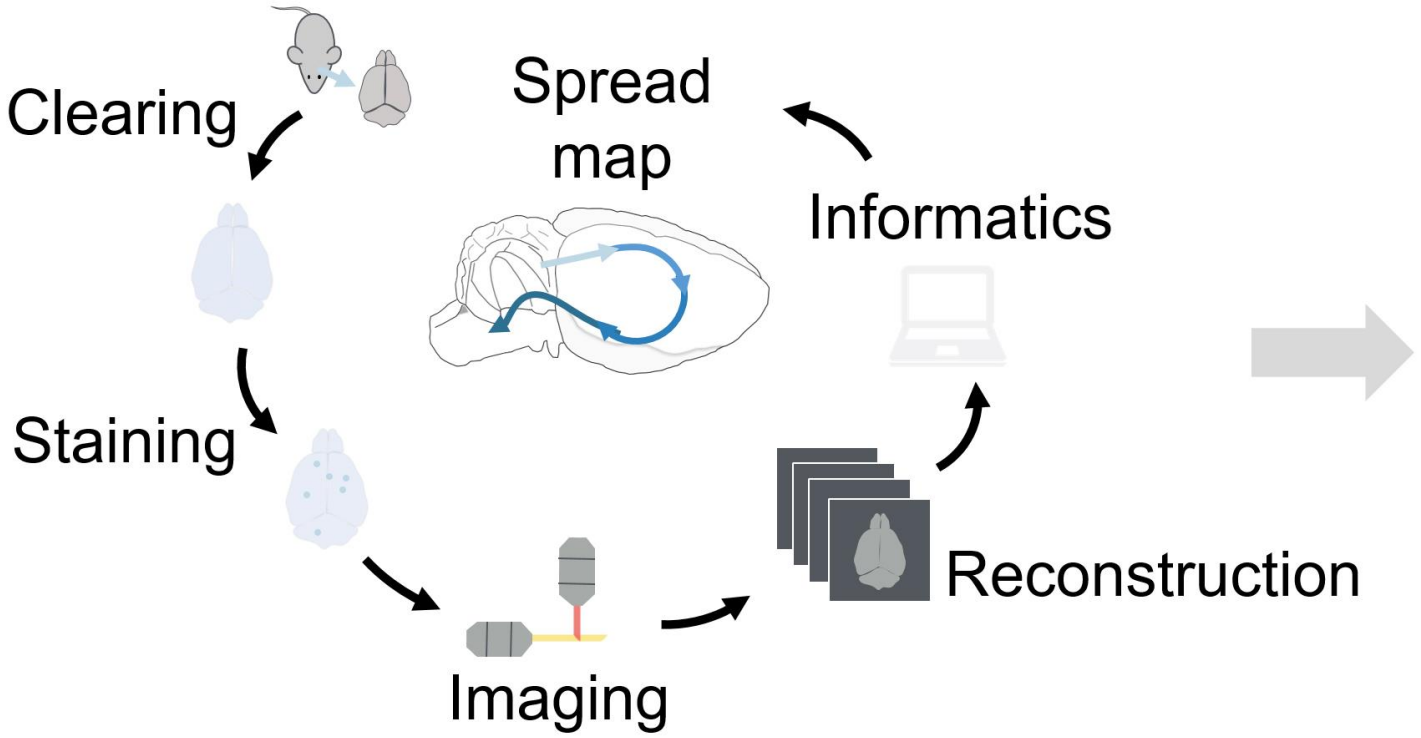


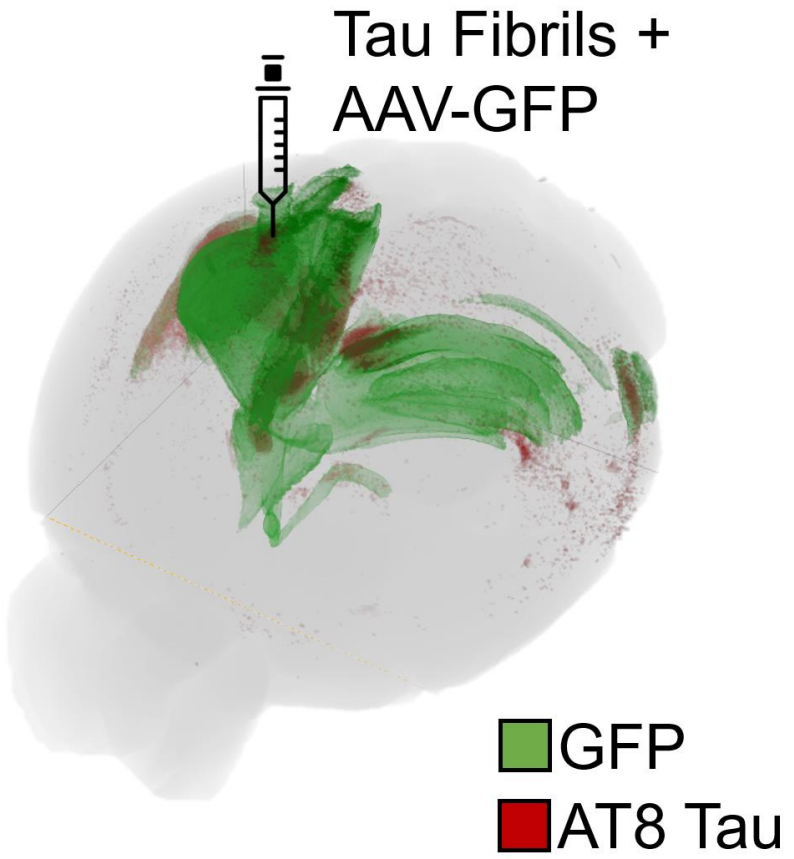
EGAMI image
University of Antwerp

Miranda et al. NeuroImage. 2019.

Case II: Multi-modal *in toto* brain imaging

CBH and BIL





EGAMI*image*
University of Antwerp

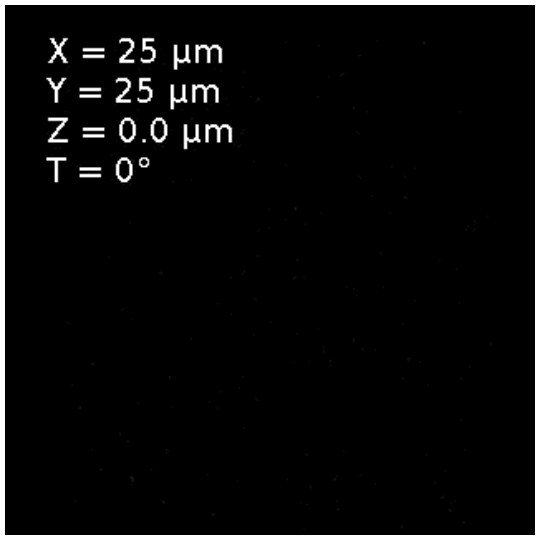
Unpublished results

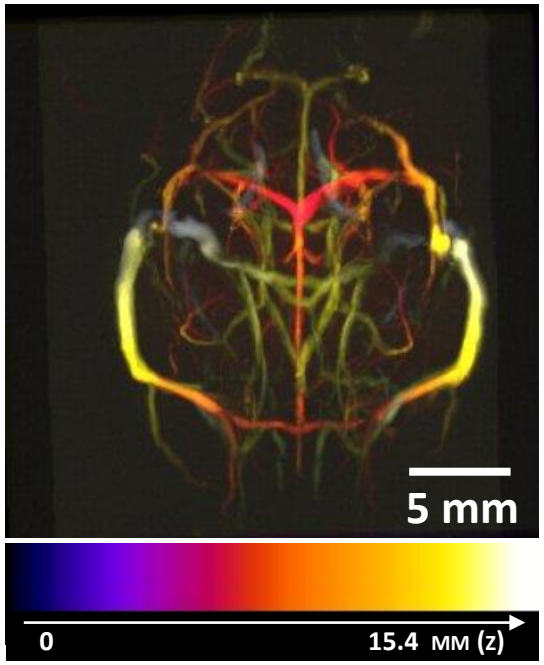
Case II: Multi-modal *in toto* brain imaging

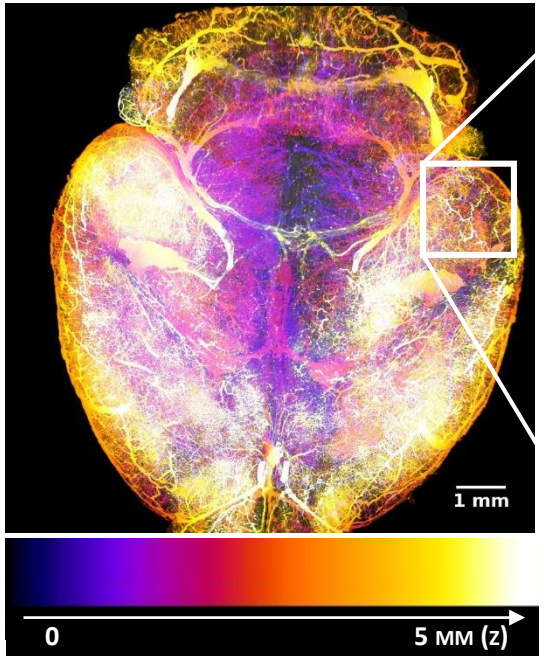
CBH and BIL

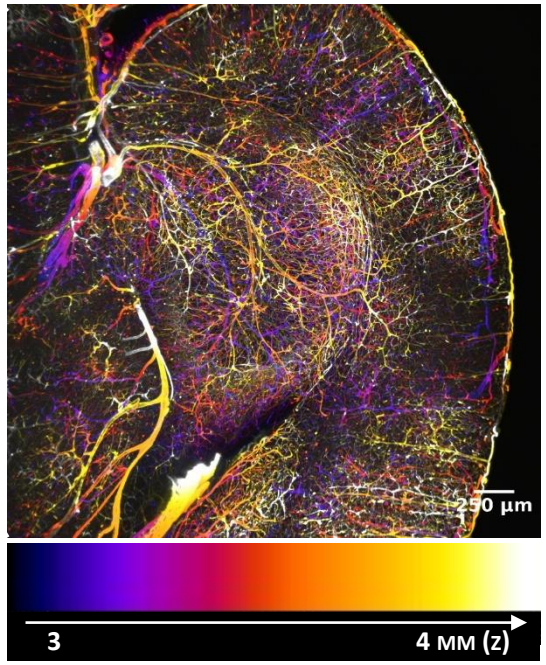
Functional MRI angiogram
- Flow speed
- Flow direction

Light-sheet imaging of cleared brain
- High-resolution anatomical data









EGAMI*image*
University of Antwerp

Unpublished results

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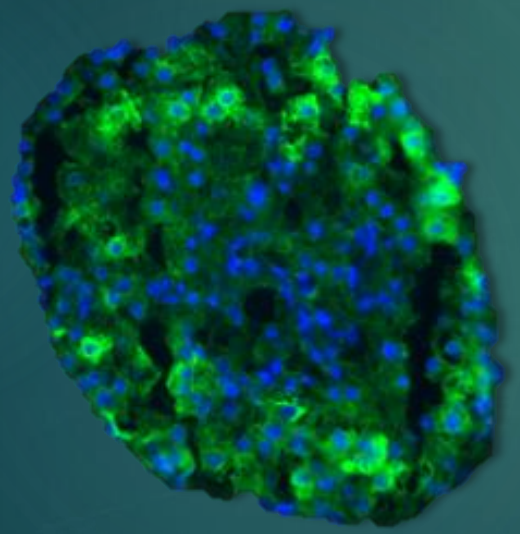
T: +32 3 265 89 82

EGAMI*image*
University of Antwerp

www.egami.be

Universiteit Antwerpen

In vitro 3D organoid model for liver disease testing



Leo van Grunsven
Liver Cell Biology research group
Vrije Universiteit Brussel (VUB),
Belgium

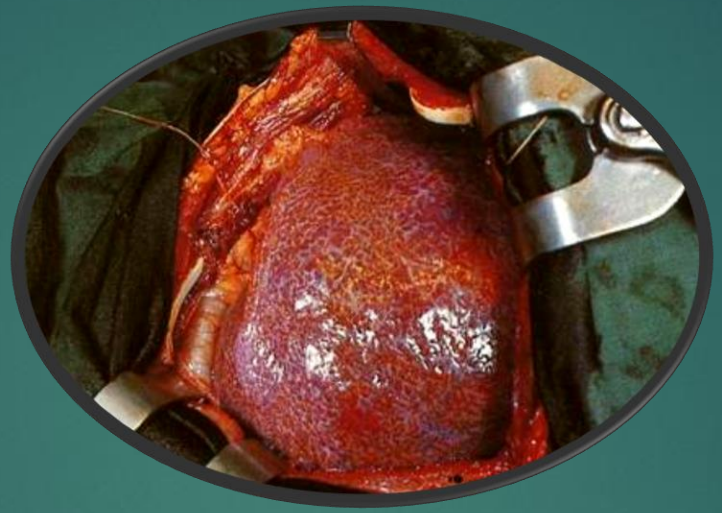
Chronic liver disease

✓6% of the EU population

✓✞ ~ 70.000/year in EU

Healthy liver

Fibrotic liver



Cirrhotic liver

No good anti-fibrotic drugs available.

Liver Transplant

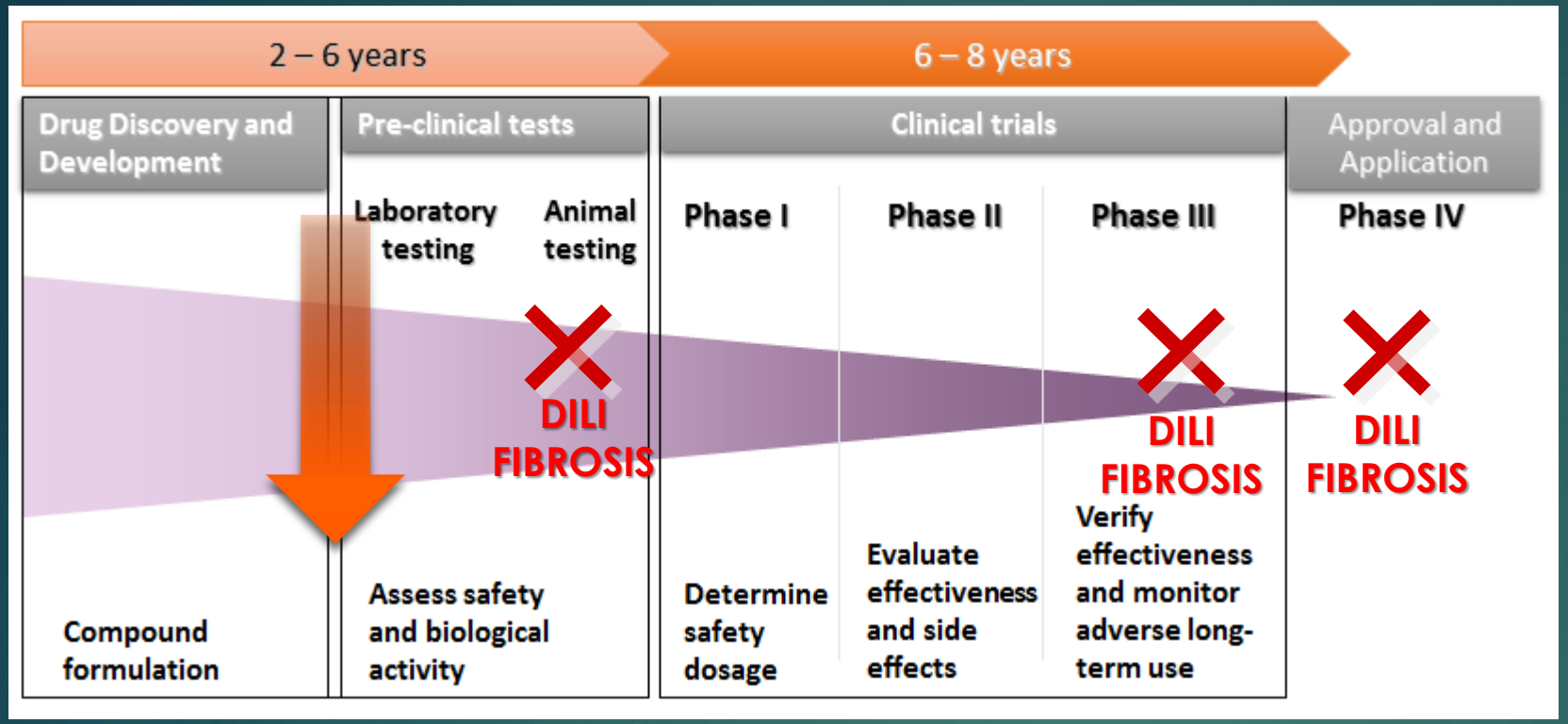
Hepatitis A,B,C,D,E

Fatty liver disease

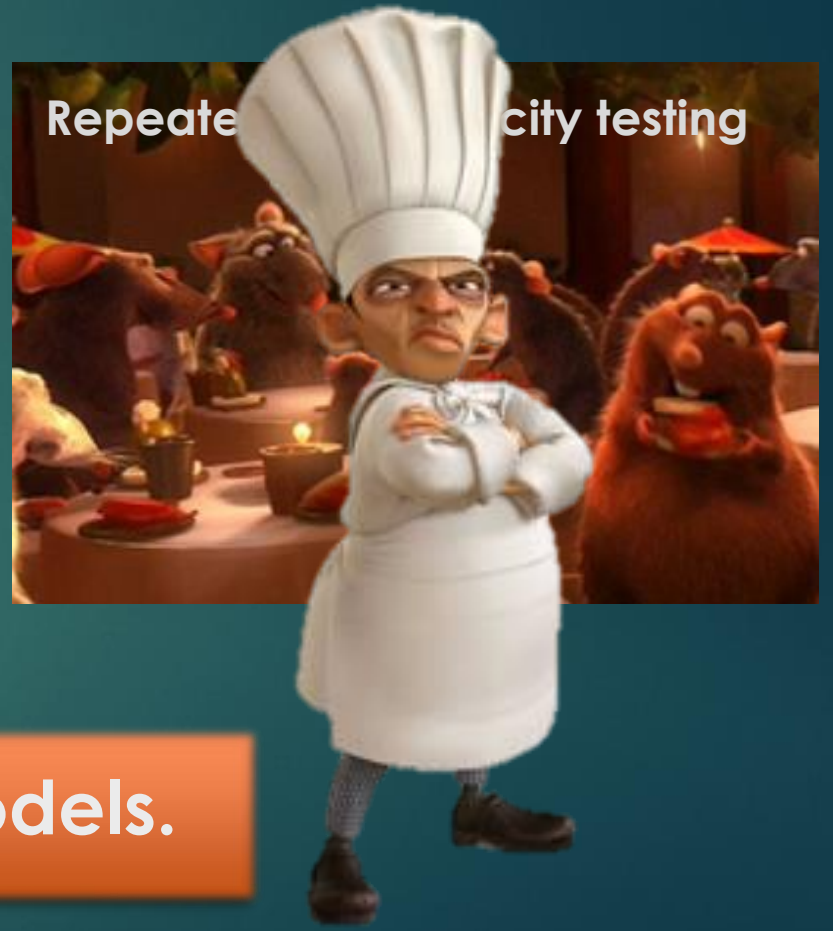
Alcohol

Drugs

Drug induced liver injury (DILI) is detected too little, too late…



Phase I - IV
Animal testing



- ± 26.2% of drug withdrawal is due to hepatic toxicity
  Fung et al., 2001, Drug Info J.

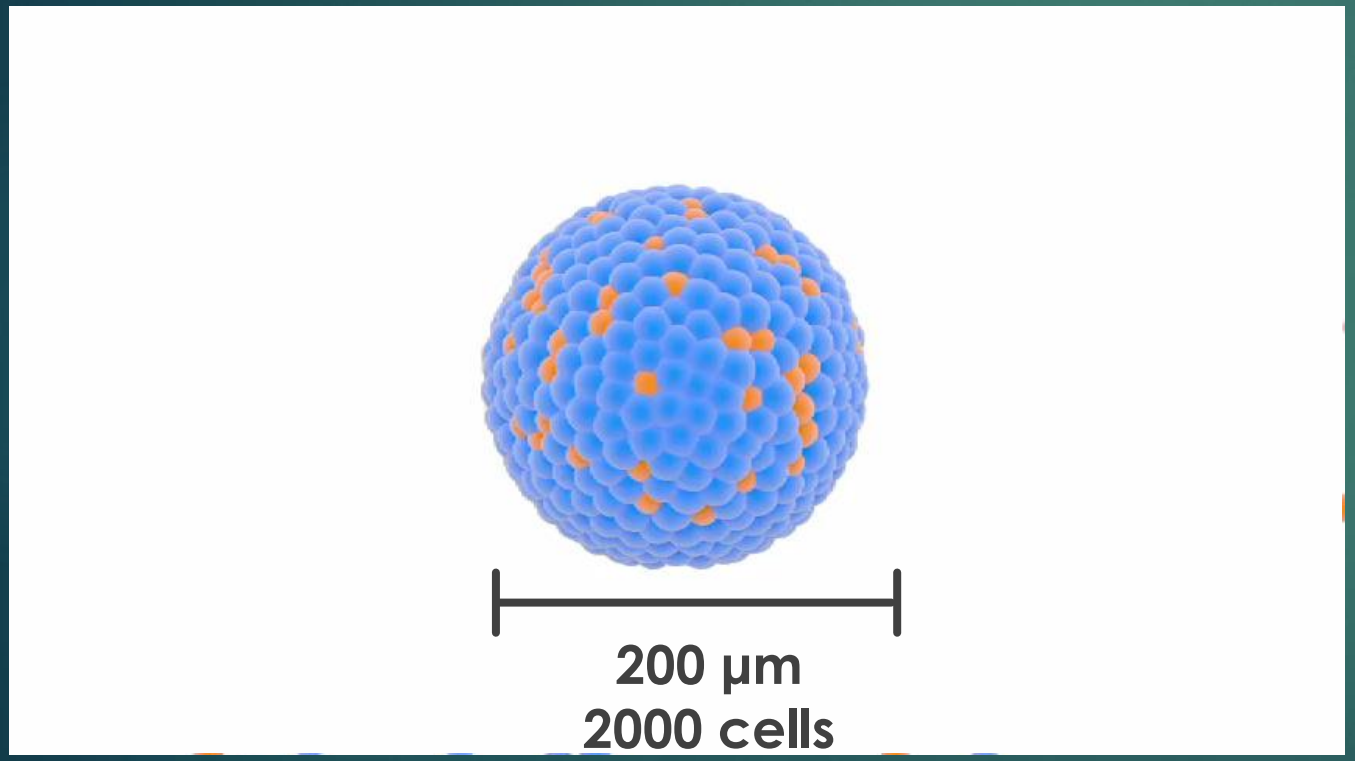
There is a need for good in vitro fibrosis models.

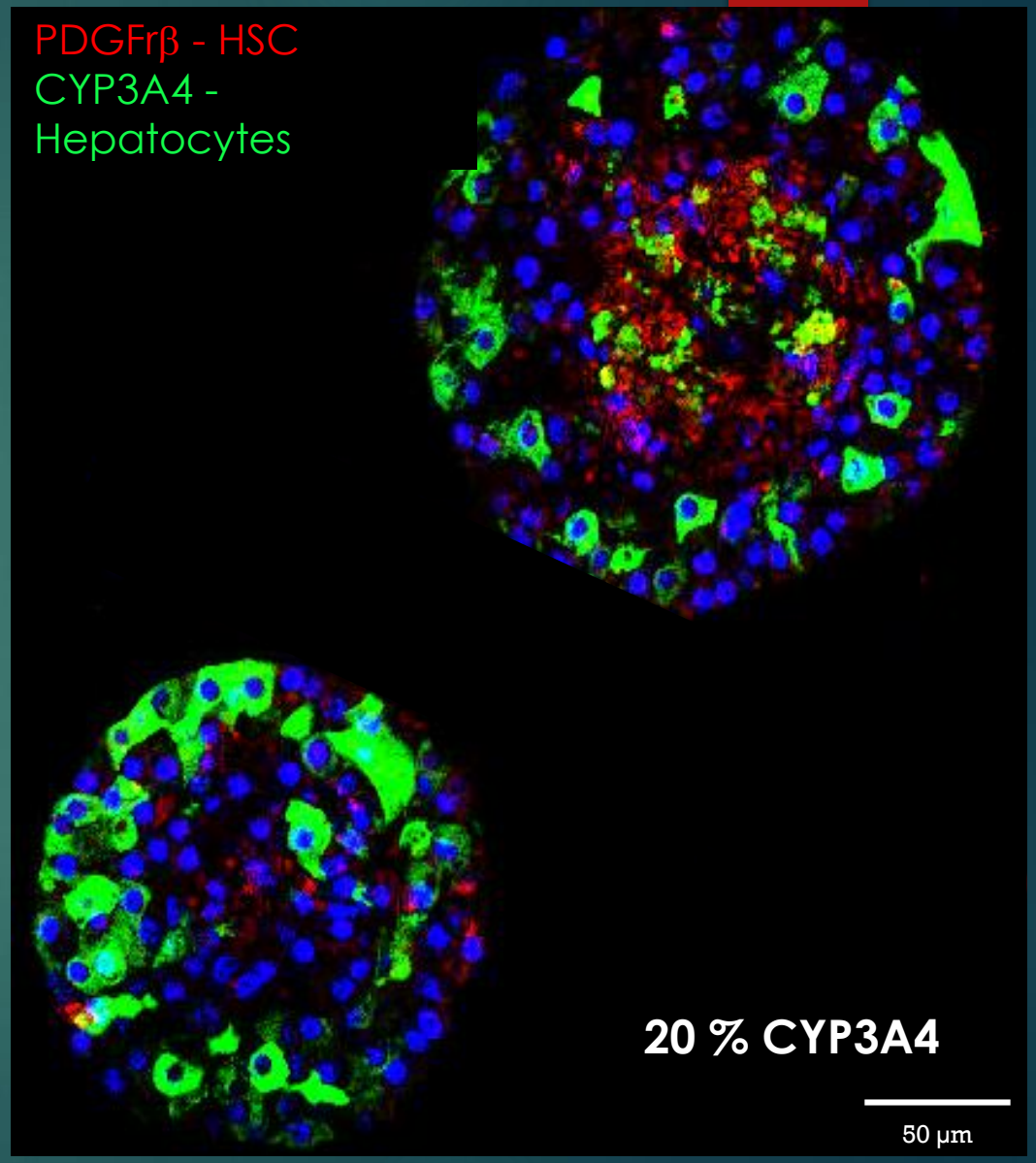
Adapted from: Matisson & Faye *Sex Differences in drug development*, Blickpunkt der Mann 2008

Human hepatic organoids

HSC: Human primary HSCs (in house:>Passage 4)
Hepatocyte: Differentiated HepaRG (Biopredic)

2 HSC : 1 Hepatocyte

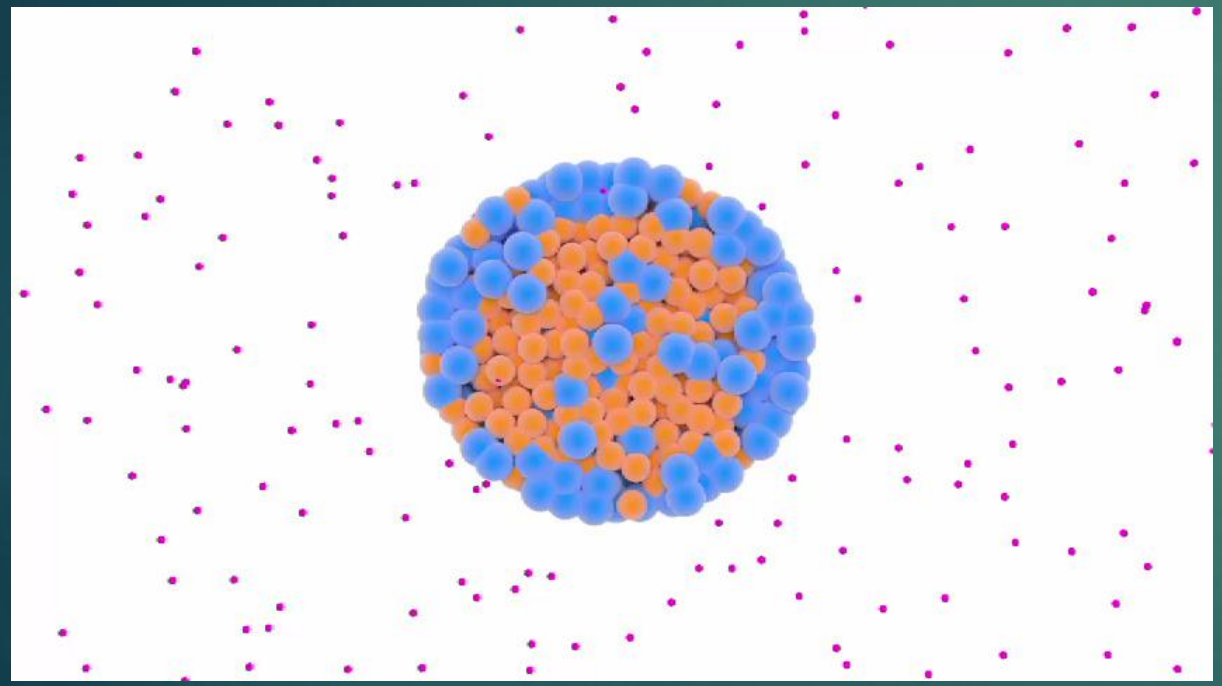




3D liver in vitro liver fibrosis model

- **A robust hepatocyte-damage dependent in vitro fibrosis model**

HepaRG/pHSC **HepaRG/iPSC-HSCs** **primary mouse Hep/HSC**



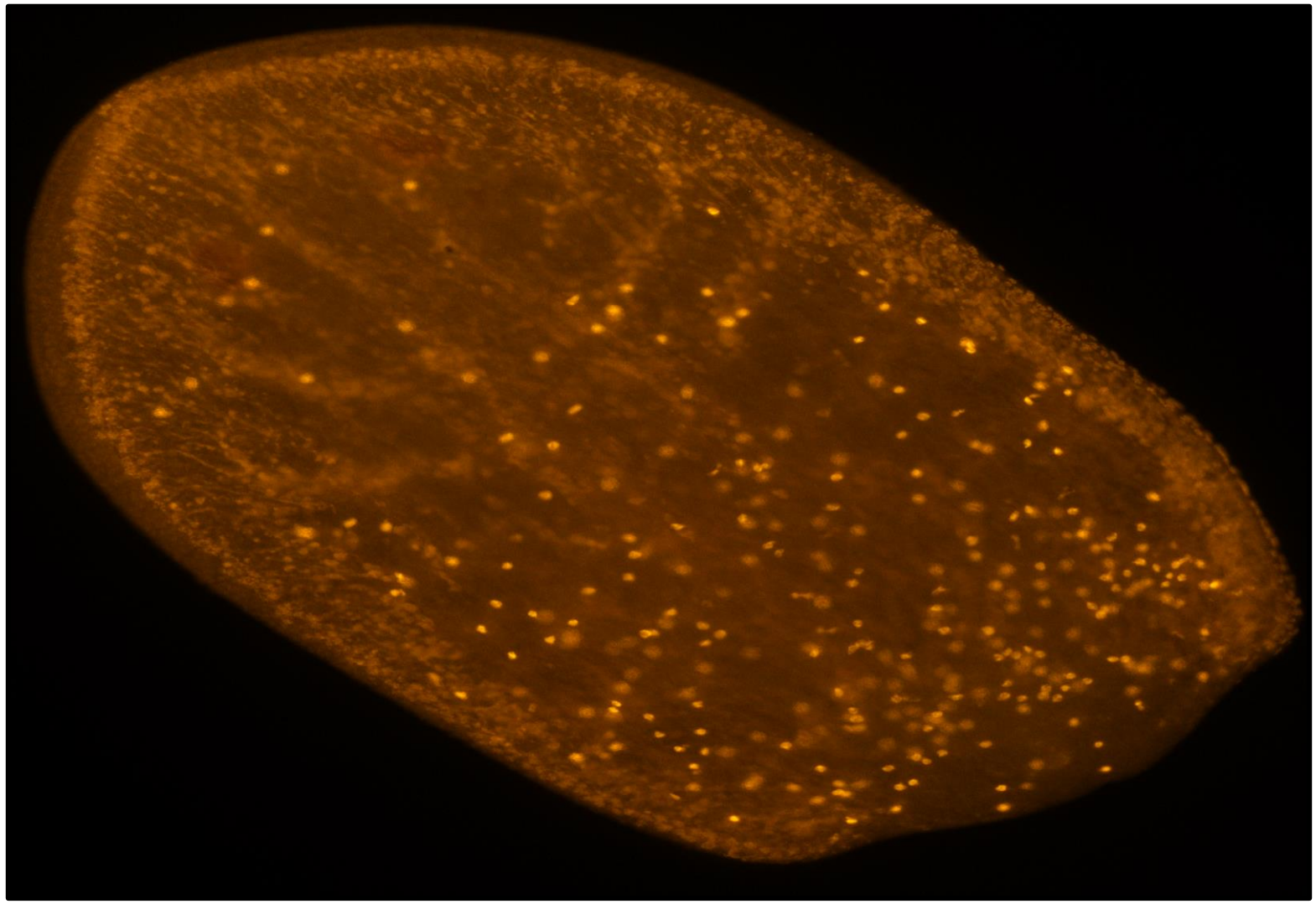
- ***Hepatocyte functional (>21 days)***
- ***HSCs quiescent***
- ***Responds to pro-fibrotic compounds***

Suitable for:

- ***Screening of pro-fibrotic compounds***
- ***Screening of anti-fibrotic compounds***
- ***Discovery platform for new targets***
  - ***Fibrosis***
  - ***Non-alcoholic fatty liver disease***

Carcinogenic screening with planarian stem cells

Kathleen Smolders, PhD
business developer
UHasselt - CMK



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Regulations

1959

stricter regulations on animal use

1986

European directive

2007

REACH by ECHA

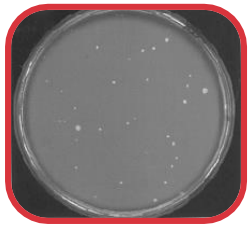
2013

Full **ban** on animal testing for cosmetics

2016

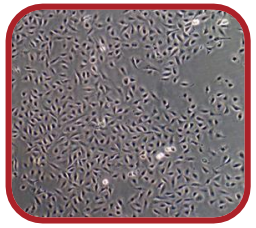
1949

Rodent assay



1970s

Ames test



1999

Cell transformation assays

?

2016

Alternatives

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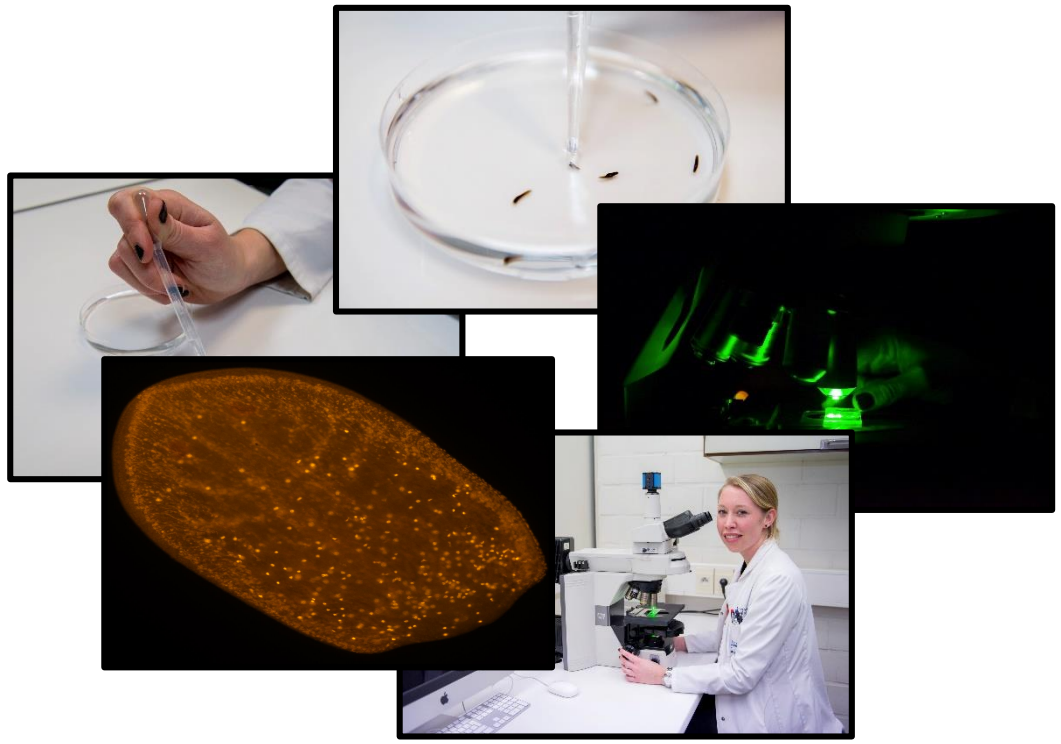
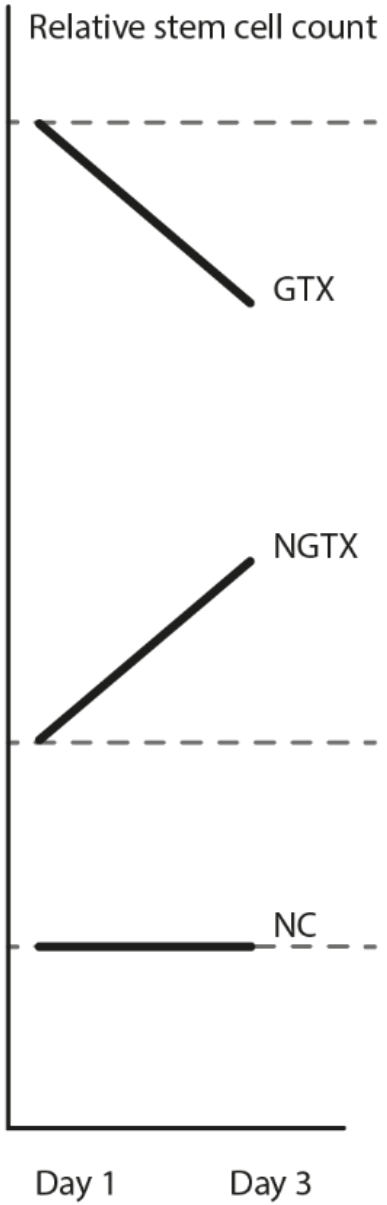
Flatworm

Adult
Pluripotent
Stem cell
In vivo

30%

Stem cell proliferation assay: simple & cost effective





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KEY FEATURES

ADVANTAGES

- **Pre-screening** of both **genotoxic** and **non-genotoxic carcinogens**
- **Cheap** model for carcinogenicity testing
- **Easy** and **fast** testing method
- ***In vivo*** method
- 30 compounds are **validated**, with a correct classification of 80%

MARKET POTENTIAL

- Prescreening of **chemicals** in risk assessment (REACH and equivalents worldwide)
- Genotoxic and non-genotoxic **carcinogens**
- **Neurotoxicity**
- **Developmental toxicity screening**
- 3R-concept: Reducing, Refining and Replacing of **animal usage**

OPPORTUNITY

Patent application available for licensing:

WO2016 146620 A1

EP15159158.3

Collaboration to further validate this assay, by contract testing or joint projects

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Contact: Kathleen Smolders, kathleen.smolders@uhasselt.be - +32 11 268542

Imec-Visionlab

FLEXRAY

Presented by **Wouter Van Putte**, PhD – 2019

imec

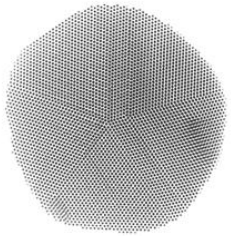
Universiteit Antwerpen

ASTRA TOOLBOX - www.astra-toolbox.com

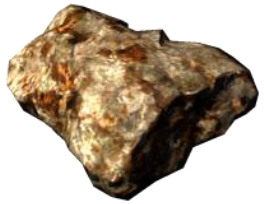
security

agro-food

electron tomography



atomic resolution



mining

fruit quality control

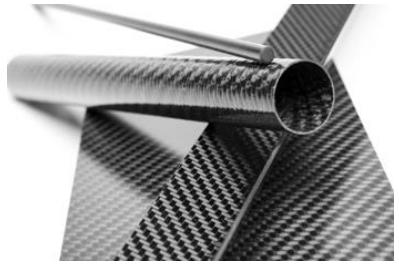
3D printed materials

diamond

inspection

automotive

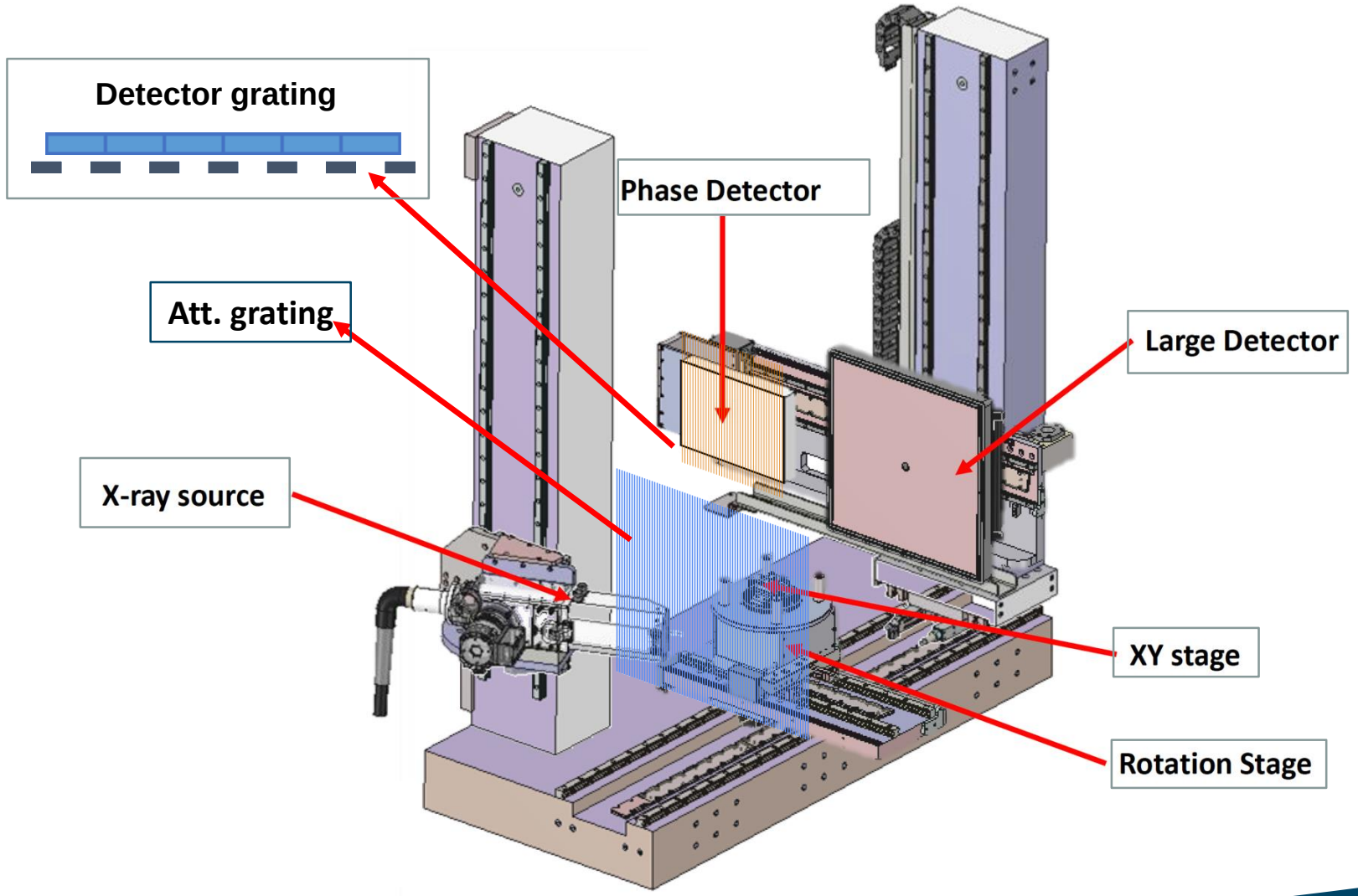
medical devices



composites

foam

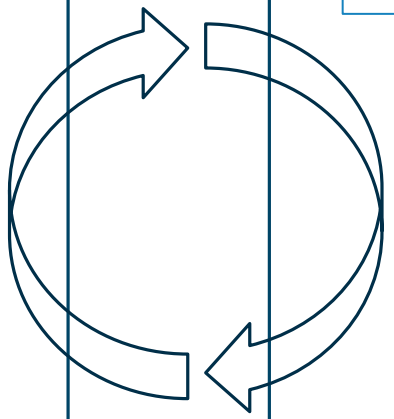
FLEXRAY: an ultra-flexible X-ray CT scanner

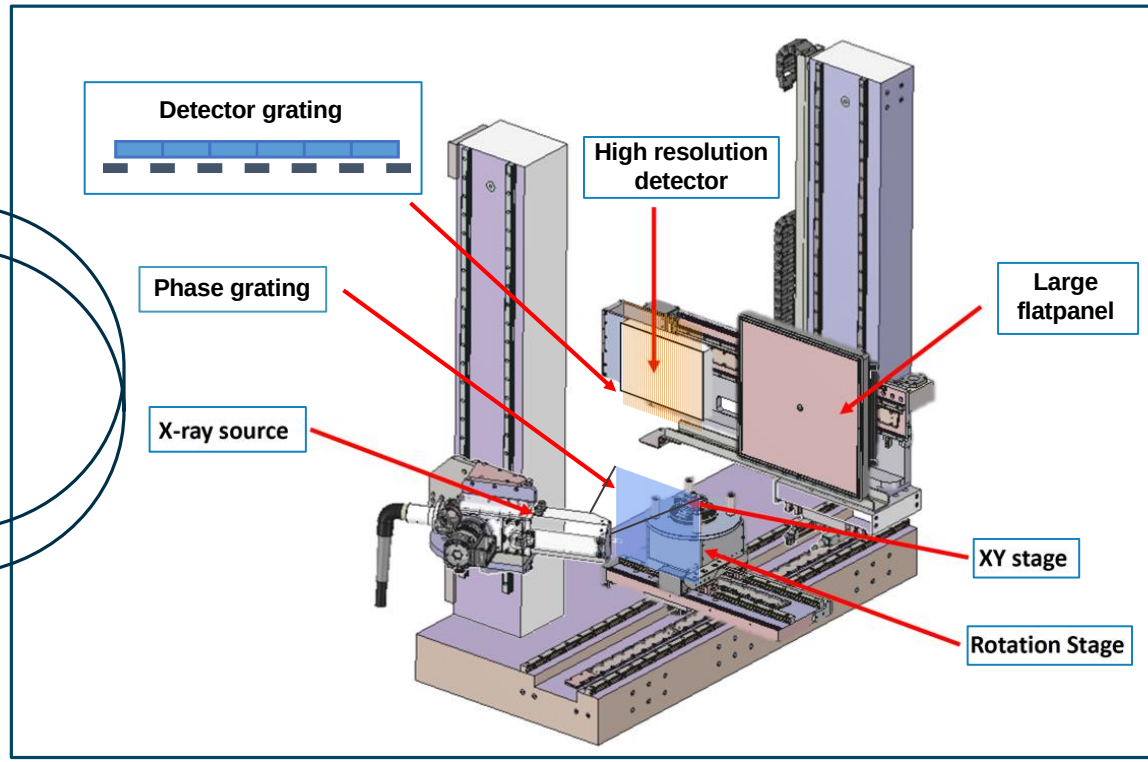


Flexray: a testplatform for industrial applications

The ASTRA Toolbox is a MATLAB and Python toolbox of high-performance GPU primitives for 2D and 3D tomography.

- **Parametric reconstruction methods for non-standard acquisition geometry**
- **Quantitative phase contrast imaging**
- **4D tomography**





ASTRA Toolbox

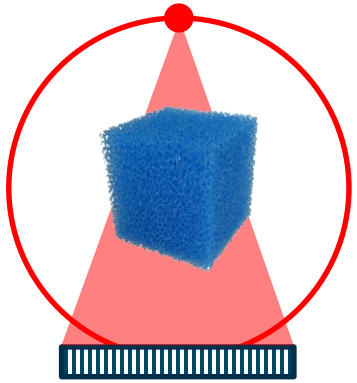
Reconstruction methodology

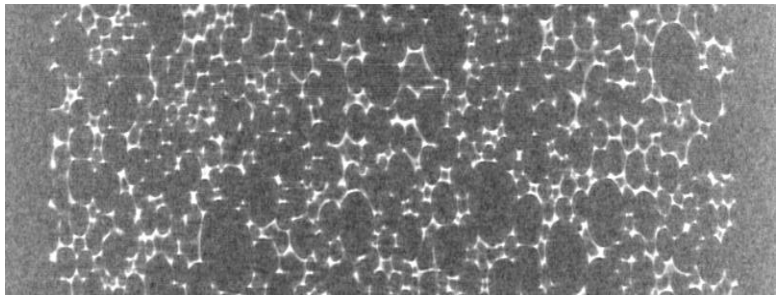
Feedback loop

Flexray

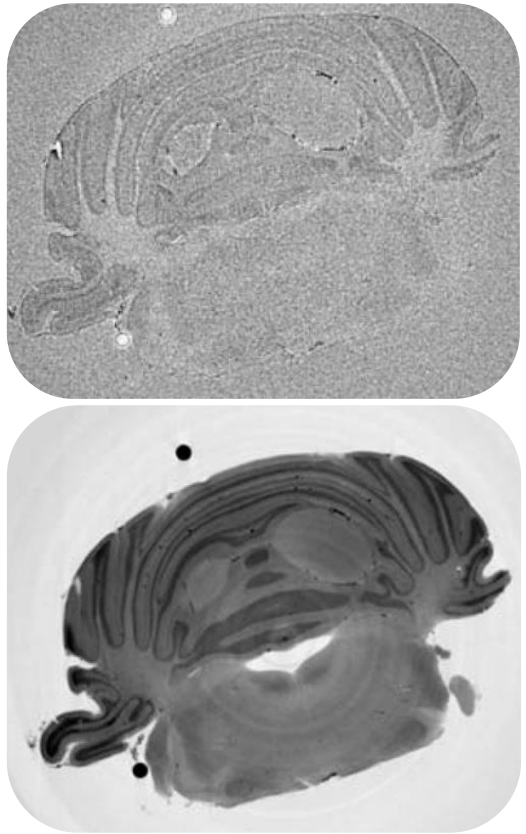
Flexible absorption and phase contrast hardware

Flexray: a testplatform for industrial applications

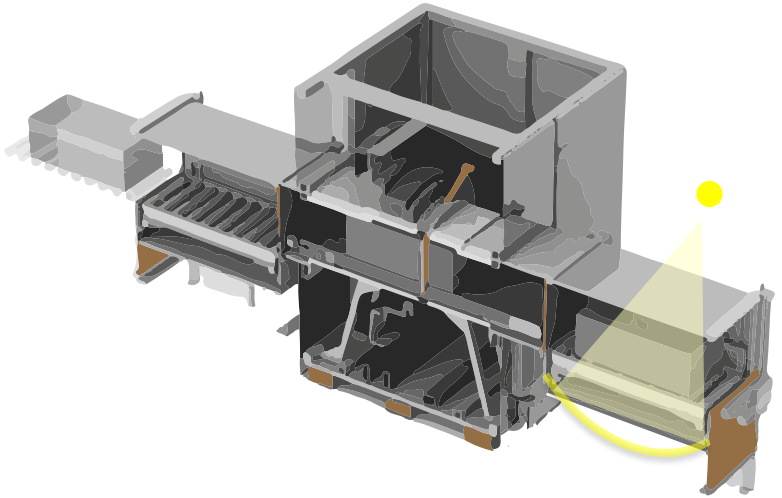




4D CT



Phase Contrast CT (*)

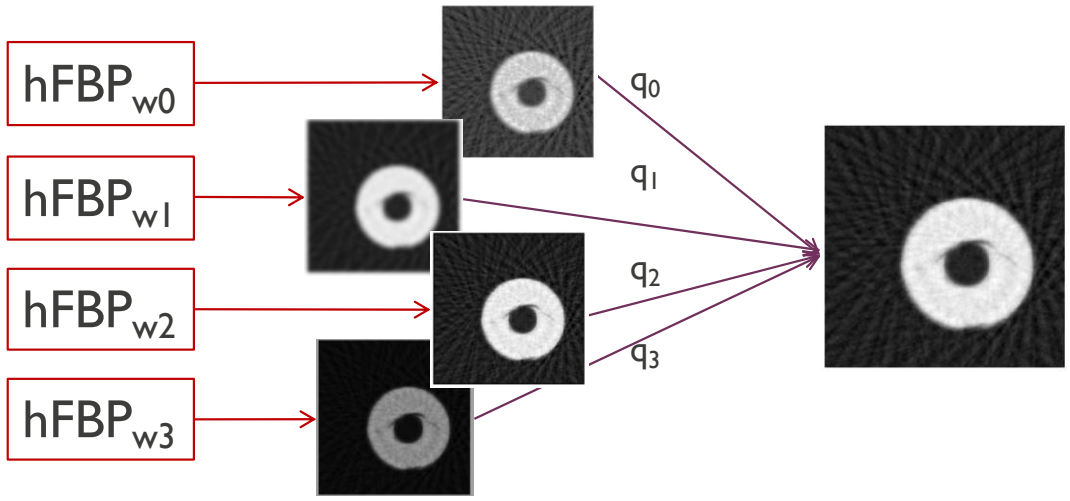


Inline XCT

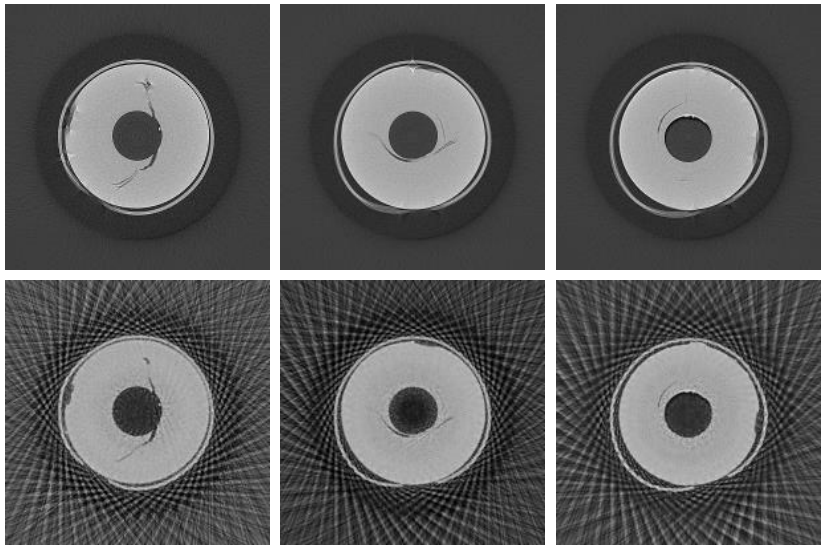
* *Courtesy of F. Pfeiffer*

Inline X-ray inspection

An automated system for ML-based QUALITY CONTROL using 3D CT information



Neural network-based reconstruction method



Top row: FBP Fan-beam reconstructions 1500 projections
Bottom row: NN-hFBP Fan-beam reconstructions 50 projections

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Miniature photonics spectrometer for real-time cancer diagnosis

Heidi Ottevaere

March 19th 2019

https://youtu.be/f-BjEDBV0uI

VRIJE UNIVERSITEIT BRUSSEL

B-PHOT BRUSSELS PHOTONICS

InSPECT

HORIZON 2020

A broadband, integrated spectrometer device for spectral tissue sensing

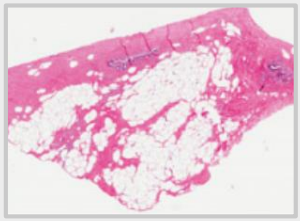
InSPECT

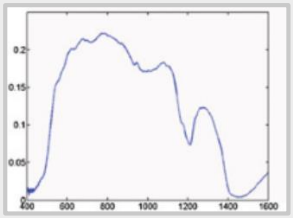
HORIZON 2020

APPLICATION RESEARCH

Medical Screening & Diagnostics

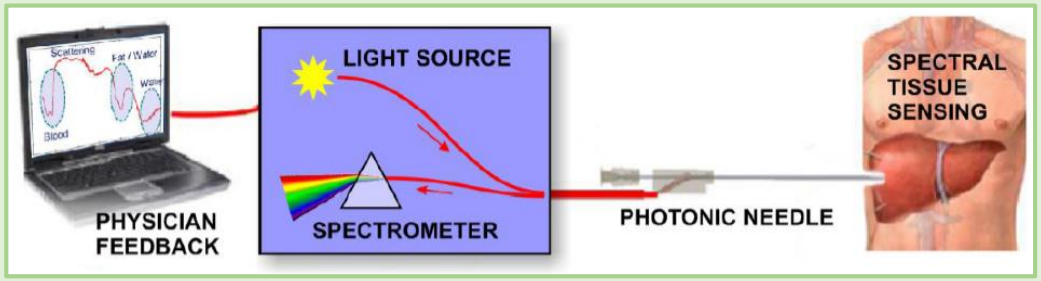
Philips & Clinical Partners





SYSTEMS RESEARCH

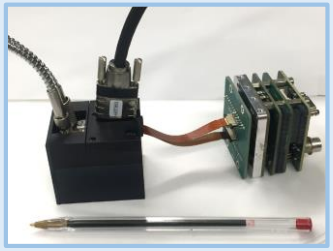
Spectral Tissue Sensing



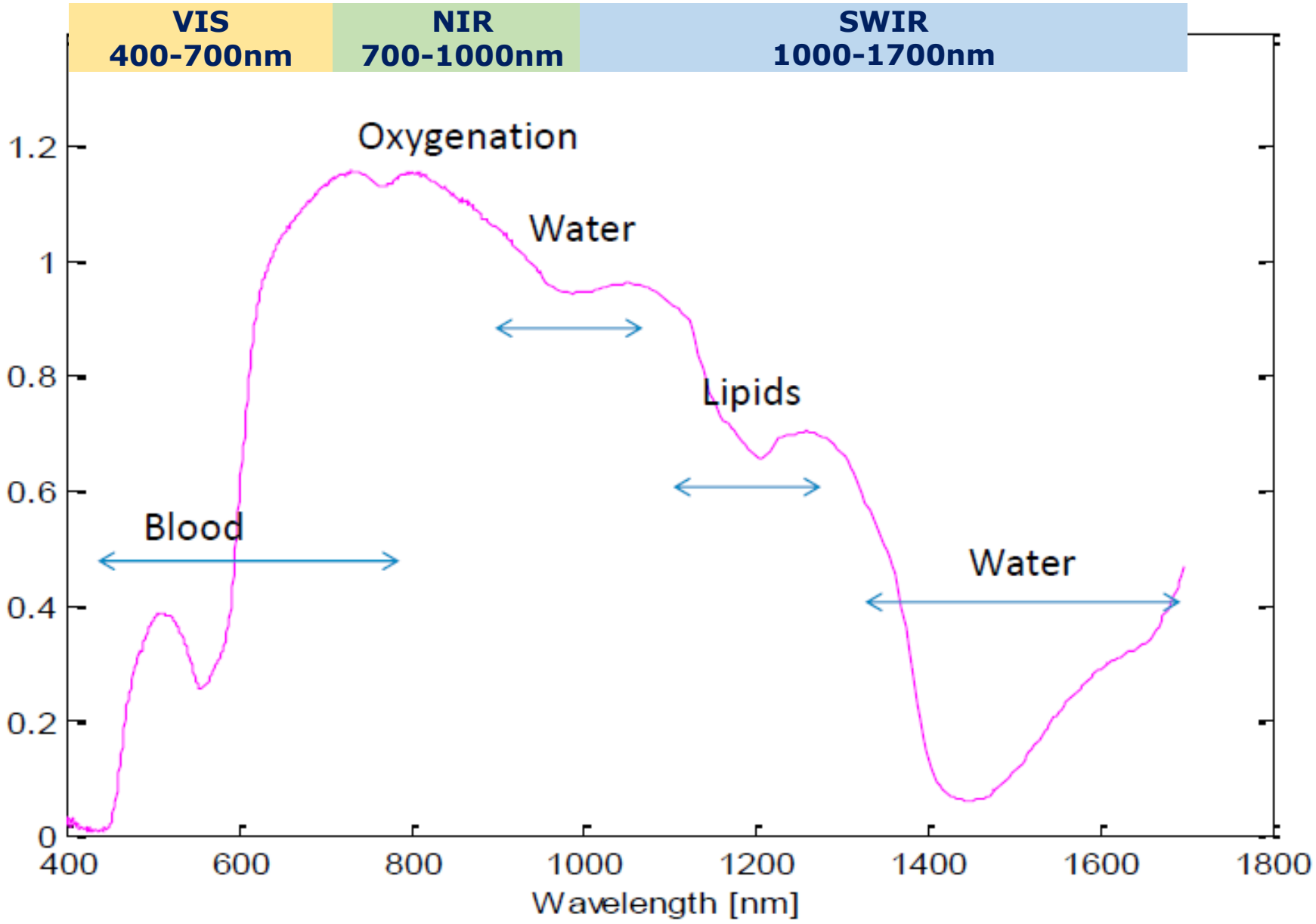
KEY ENABLING TECHNOLOGY

Miniature Spectrometer

1 inch³ VIS-IR spectrometer



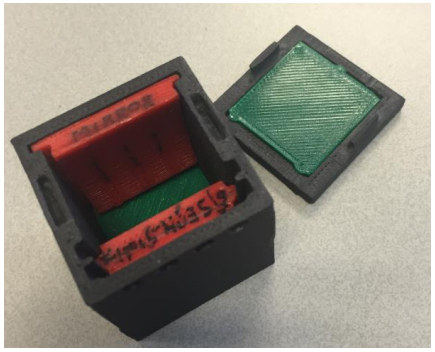
Diffuse Reflectance Spectroscopy



Perspectives

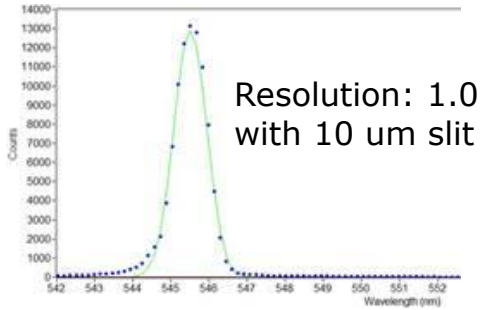
SpectroBlocks

❖ ***Towards high-volume production: "cubic inch"***



❖ ***Towards miniature size & low-cost: "cubic cm"***

Modular, free sensor choice



Resolution: 1.0 nm
with 10 um slit