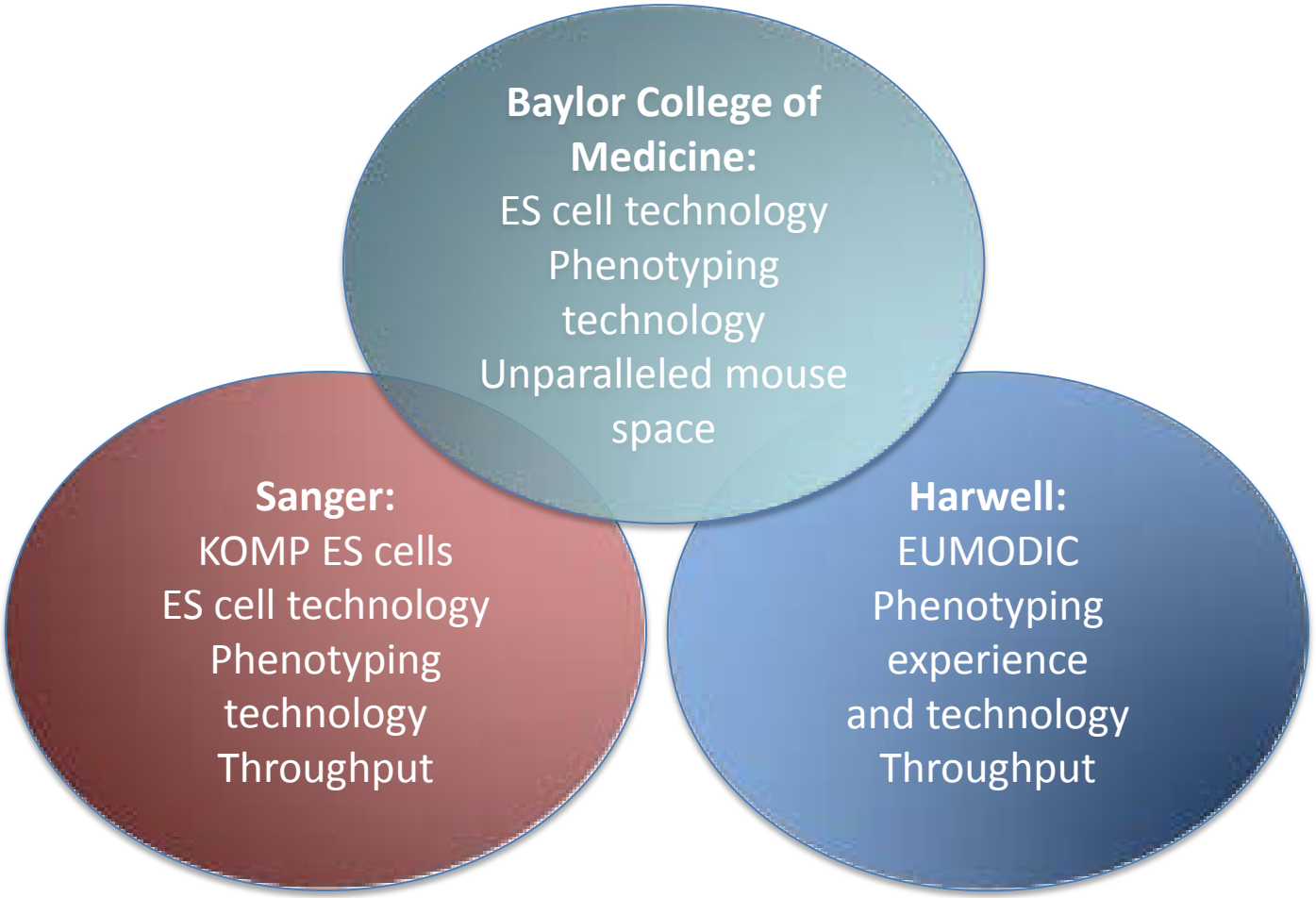
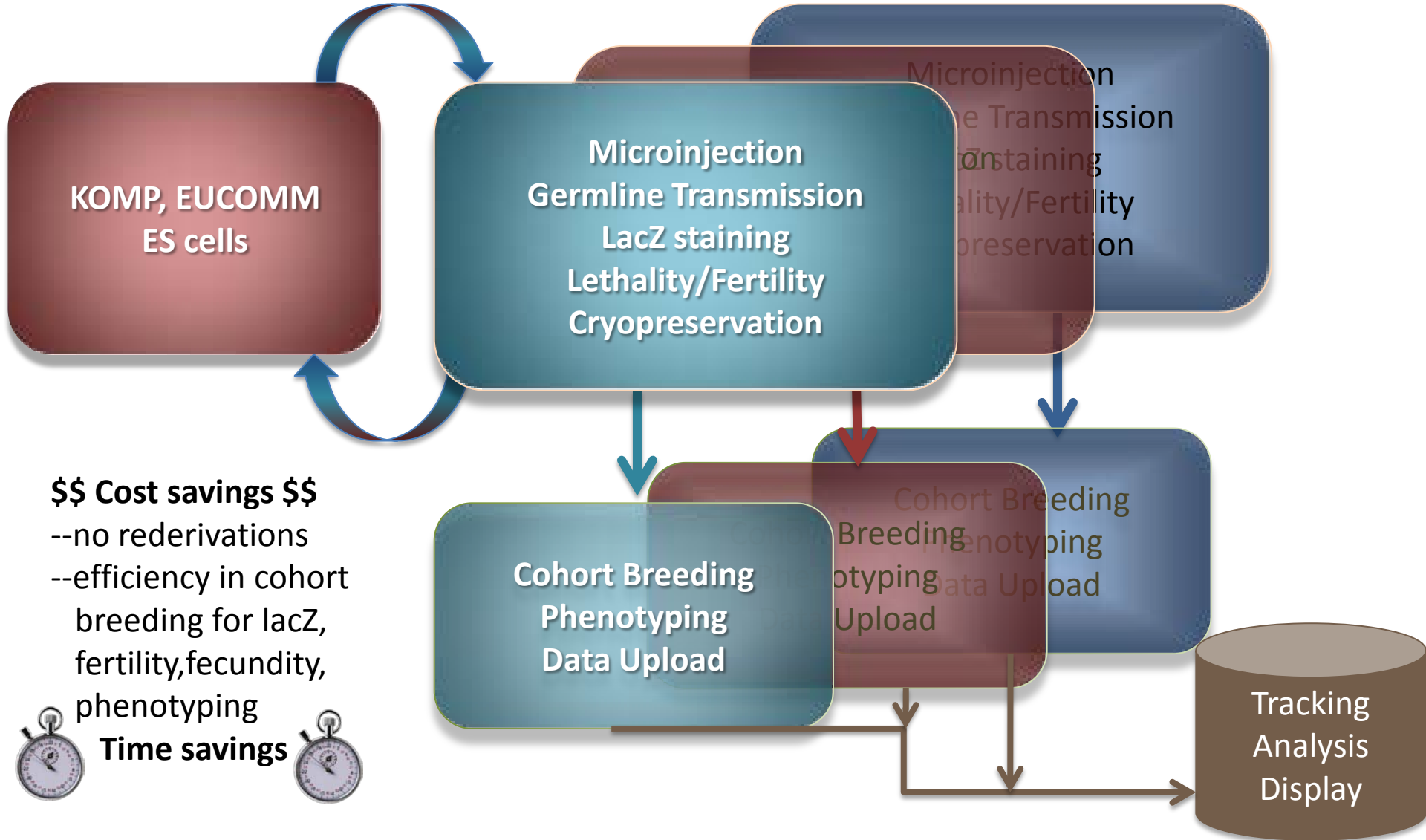
KOMP^2: the BaSH consortium

Monica Justice and Rich Paylor
Baylor College of Medicine
Houston, TX

BaSH: experience translates into throughput



BaSH: Mouse Production linked with Phenotyping for KOMP^2



$$ Cost savings $$

--no rederivations

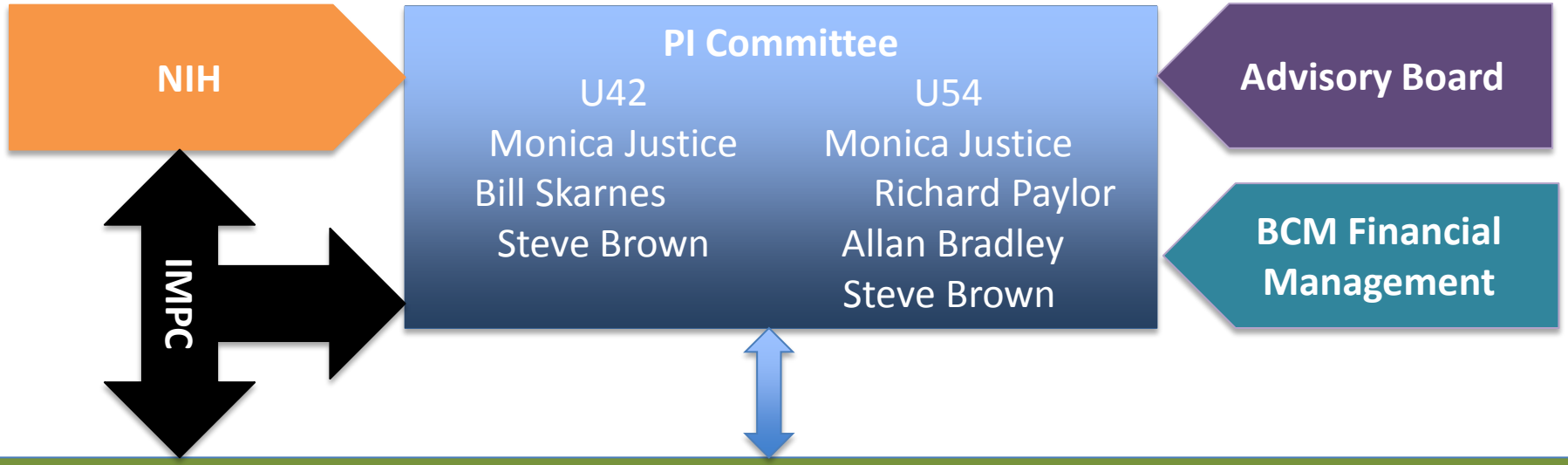
--efficiency in cohort breeding for lacZ, fertility,fecundity, phenotyping

Time savings

Scientific Leadership and impact

- **BaSH** is comprised of ***THREE*** leading institutions
- ES cell and gene targeting technology has a strong foundation at Baylor
  - Unparalleled mouse infrastructure
  - Embedded within a medical school
  - Clinical and translational applications
- Sanger has brought genomics to mouse genetics
- Harwell has > 60 year history of mouse genetics
- KOMP and EUCOMM mice now constitute majority [80%] of alleles distributed

Coordination/Cooperation



Operations Committee

U42

Bill Skarnes
Franco DeMayo
Tom Weaver

Allan Bradley, Austin Cooney, Cindy Buckmaster
Ramiro Ramirez-Solis, Sarah Wells, Martin Fray,
Ann-Marie Mallon, Bin Liu

U54

Richard Paylor
Allan Bradley
Tom Weaver

Steve Brown, Corey Reynolds, Ramiro Ramirez-Solis
Jacqui White, Mary Dickinson
Ann-Marie Mallon, Bin Liu

Sanger Institute : A record of production

- Allan Bradley, Bill Skarnes, Ramiro Ramirez-Solis
- Major player in KOMP[1]
  - 5,000 conditional alleles + targeting vectors
- Major player in EUCOMM
  - 5,346 alleles to date, towards a total of 8,500
- MirKO, microRNA resource
- Vector production > 12,800 vectors
- 400+ mouse lines

Sanger Institute: ES cell resources

- KOMP and EU resource centers are a risk factor for KOMP^2
- Distribution centers overwhelmed by demand
  - Backlog can affect throughput
- Sanger holds original archival copies of:
  - Majority of clones produced: CSD, EUCOMM, MirKO
  - All conditional targeting vectors and intermediates
  - All data supporting vector and allele construction
- Sanger will implement stringent allele QC

Sanger Institute: Technology developer

- High throughput recombineering
- Computational allele design
- JM8 and JM8 Agouti cell lines
- Anitrack – internal mouse tracking data base and electronic “health record” of more than 400,000 mouse citizens
- C57BL/6N – Cre and Flp deletor lines
- IT
  - KERMITS, iMITS

MRC Harwell: National Centre of Excellence in Mouse Genetics from 1950

- MRC Mission

  Improve human health through *world-class medical research*

International Renown in Mammalian Genetics

- Impact of Radiation on Genomes
- X Chromosome Inactivation & Imprinting
- Frozen Embryo Bank
- Mouse Models of Disease
- Systematic Mutagenesis & Phenotyping

Mammalian Genetics Unit (MGU)

- Genetics and functional genomics research into a wide variety of disease models
- 10 Research Programs
- Steve Brown, Director

Mary Lyon Centre

- National Infrastructure for Mouse Genetics
- Vivarium; 4,000 m2; 14,000 IVC Cages, max capacity 52,000 mice
- National Biorepository & Distribution Centre
- Tom Weaver, Director

MRC-MLC Harwell

1. Scientific Leadership
   - Provide Expertise
   - Influence National Funding Programs

2. Operational Engine
   - Infrastructure to Deliver Capability and Quality

3. Resource Portal To Mouse Community
   - Open Access to Mice, Data, Analysis Tools

European Mouse Programmes

- EUCOMM - European Conditional Mouse Mutagenesis
  - Developing mouse mutants for most of the genes in the mouse genome
- EUMORPHIA - European Mouse Phenotyping
  - Development and standardisation of mouse phenotyping platforms
- EUMODIC - European Mouse Disease Clinic
  - Undertake a major pilot programme to utilise standardised phenotyping platforms for the analysis of mutants from EUCOMM
- EMMA - European Mouse Mutant Archive
  - Archiving and dissemination of mice
- InfraFrontier - European Infrastructure Network
  - Preparing European Infrastructure for phenotyping and archiving

Baylor College of Medicine

- Monica Justice, Franco DeMayo, Richard Paylor
- Top medical school
- Genetics department ranked in top 10
- Unparalleled mouse facilities
  - Operated by Center for Comparative Medicine
  - 115,000 cages, 4.4 acres
  - New TMFT facility free of all pathogens
    - Helicobacter and MNV
  - Techniplast Green line ventilated cages
  - Techniplast robotic cage washers
- IT Infrastructure, Mouse ES cell and GEM Cores, Mouse Phenotyping and Behavior Cores

History of collaborative interactions

- Numerous EU projects
  - EMMA, EUCOMM, EUMODIC, EUCOMMTools
- KOMP CSD [CHORI-Sanger-UC Davis]
- Strong interaction among PIs
  - Baylor/Sanger : Justice-Bradley
  - Sanger/Harwell : Brown-Weaver-Bradley-Skarnes
  - Harwell/Baylor : Brown-Justice
- KOMP^2 preparation

Benefits of BaSH consortium

- International cooperation and coordination
  - Ongoing, proven to work in EUMODIC
  - Required for success of IMPC
  - Paradigm for global science initiatives
- Multi-member composition
  - Elasticity
    - One member can increase capacity
    - Reduced risk
  - Cross-referencing strain values for QC
- Technology transfer
  - Genotyping platform
  - IT developments
  - Phenotyping assays
  - Encourages innovation

Work Distribution

Year	1	2	3	4	5	Total
BaSH-Production	**130**	**180**	**185**	**200**	**130**	**825**
Phenotyping	90	130	200	220	185	825
BCM-Production	**50**	**80**	**85**	**100**	**130**	**445**
Phenotyping	30	50	100	120	145	445
Sanger-Production	**40**	**50**	**50**	**50**	**0**	**190**
Phenotyping	30	40	50	50	20	190
Harwell-Production	**40**	**50**	**50**	**50**	**0**	**190**
Phenotyping	30	40	50	50	20	190

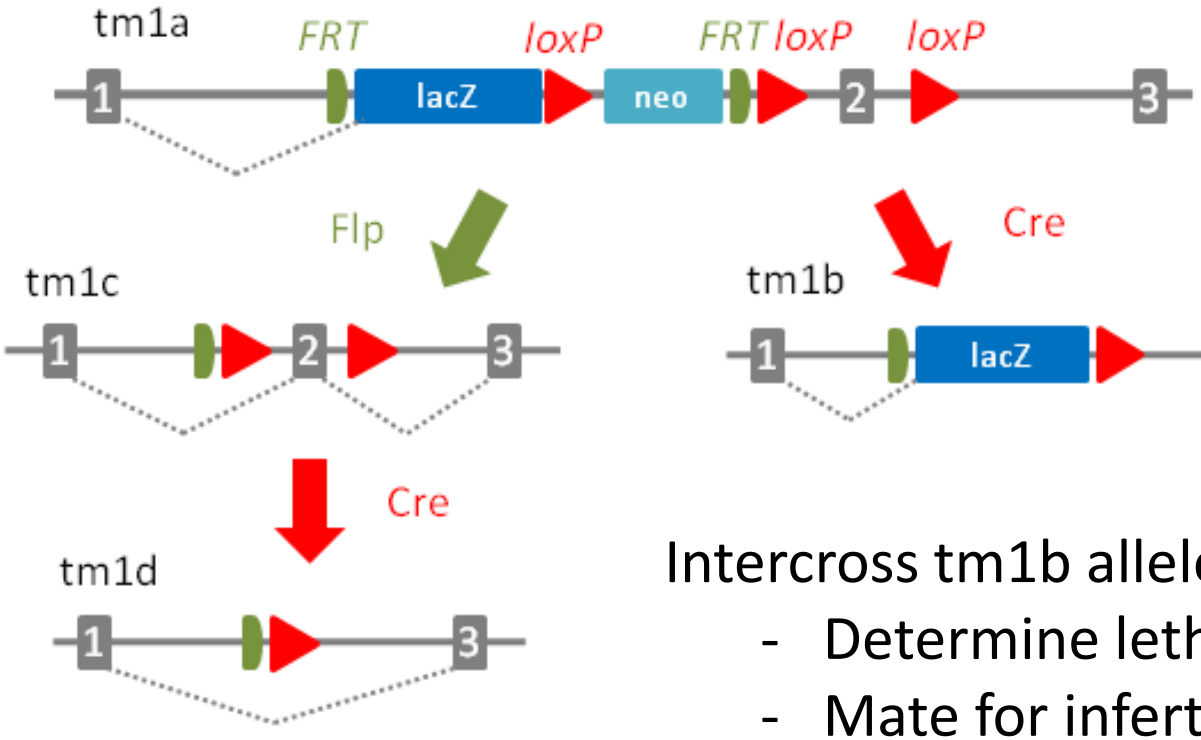
Consortium will allow ramp up

BCM will produce over ½ of mutant lines

Role of consortium will end by year 5

Preparation for next phase

Knockout First allele



Cryopreserve after Germline transmission

Cryopreserve after critical exon deletion

Intercross tm1b allele

- Determine lethal status
- Mate for infertile status
- lacZ analysis
- Homozygous or heterozygous Cohorts/littermates to phenotyping

BaSH Consortium: Broad Based Phenotyping

Overall Goals:

- Assess gene function in multiple systems
- Communicate observations and interpretations
- Develop technologies and platforms

Domains

Nervous system	Sensory
Cardiac	Respiratory
Integumentary	Skeletal
Metabolism	Immune & Blood

Assay selection

- moderate/high-throughput
- reliable & reproducible
- opportunities for ‘challenge’ assessments
- transferable technology
- ‘Easy’ statistical analysis

Pipeline Development & Implementation

Expertise

BaSH consortium better than sum of parts
Leaders in field are part of BaSH
Training: transferring protocols

Capacity

throughput, housing, etc

Assay sensitivity and variability

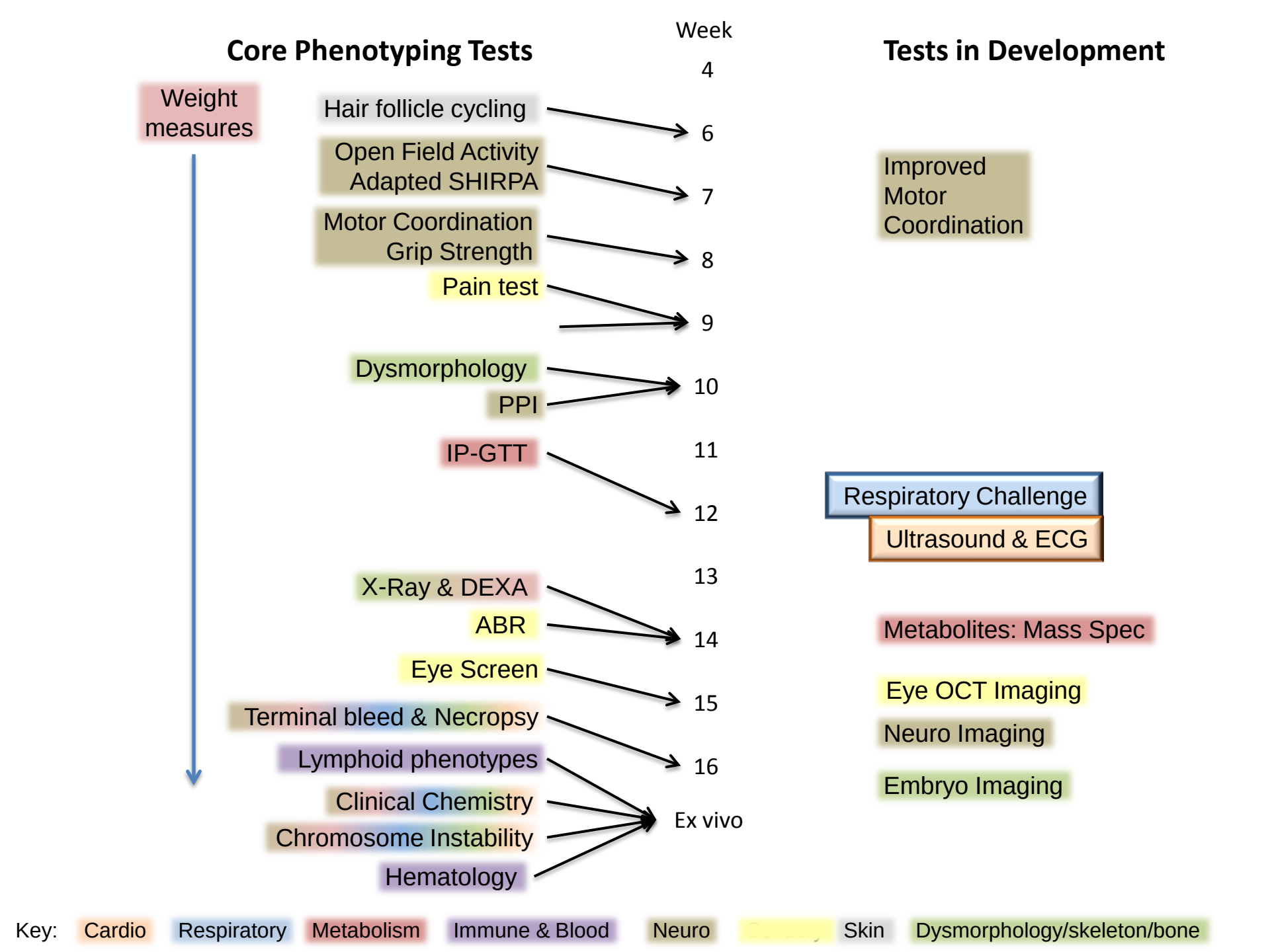
Behavior well known for challenge

Control inclusion

B6N not sufficient, need WT littermates

Analysis

Easily imported, and simplest ‘stats’ possible


Core Phenotyping Tests
Week
Tests in Development
Weight measures
Hair follicle cycling
4
6
Open Field Activity
Adapted SHIRPA
7
Improved Motor Coordination
Motor Coordination
Grip Strength
8
Pain test
9
Dysmorphology
10
PPI
IP-GTT
11
12
Respiratory Challenge
Ultrasound & ECG
13
X-Ray & DEXA
ABR
14
Metabolites: Mass Spec
Eye Screen
15
Eye OCT Imaging
Terminal bleed & Necropsy
Neuro Imaging
16
Lymphoid phenotypes
Embryo Imaging
Clinical Chemistry
Ex vivo
Chromosome Instability
Hematology
Key:
Cardio
Respiratory
Metabolism
Immune & Blood
Neuro
Skin
Dysmorphology/skeleton/bone

BCM Mouse Behavioral Testing
Rich Paylor, Director

TMF (transgenic mouse facility)

- 13 testing rooms
- 30-35 different assays available
- 12 laboratories - 35 investigators
- 8000 hrs of use

* (most of the users have moved to NRI)

Perfect timing to access state-of-the-art facility

BCM Mouse Phenotyping Core

- 3400 sq.ft state-of-the art Phenotyping facility
- 2 Full time staff members
- Corey Reynolds, Director- 8 years experience

Mouse Phenotyping Core

- Vevo 770 Ultrasounds
- Bruker 7.0T MRI (body and head imaging)
- Gamma Medica CT/SPECT
- Kodak X-Ray/Fluorescence Imager
- Piximus Bone Densitometer
- Oxymax Indirect Calorimetry (chambers/treadmill)
- Unrestrained Whole Body Plethysmography
- Metabolic Cages
- DSI Blood Pressure and ECG telemetry
- Non-Invasive Blood Pressure
- ECGenie
- Treadmills
- Mini Mitter running wheel
- Slit lamp microscope
- Full Surgical suite with several procedures

Mouse Phenotyping Core

BCM CCM Pathology Laboratory

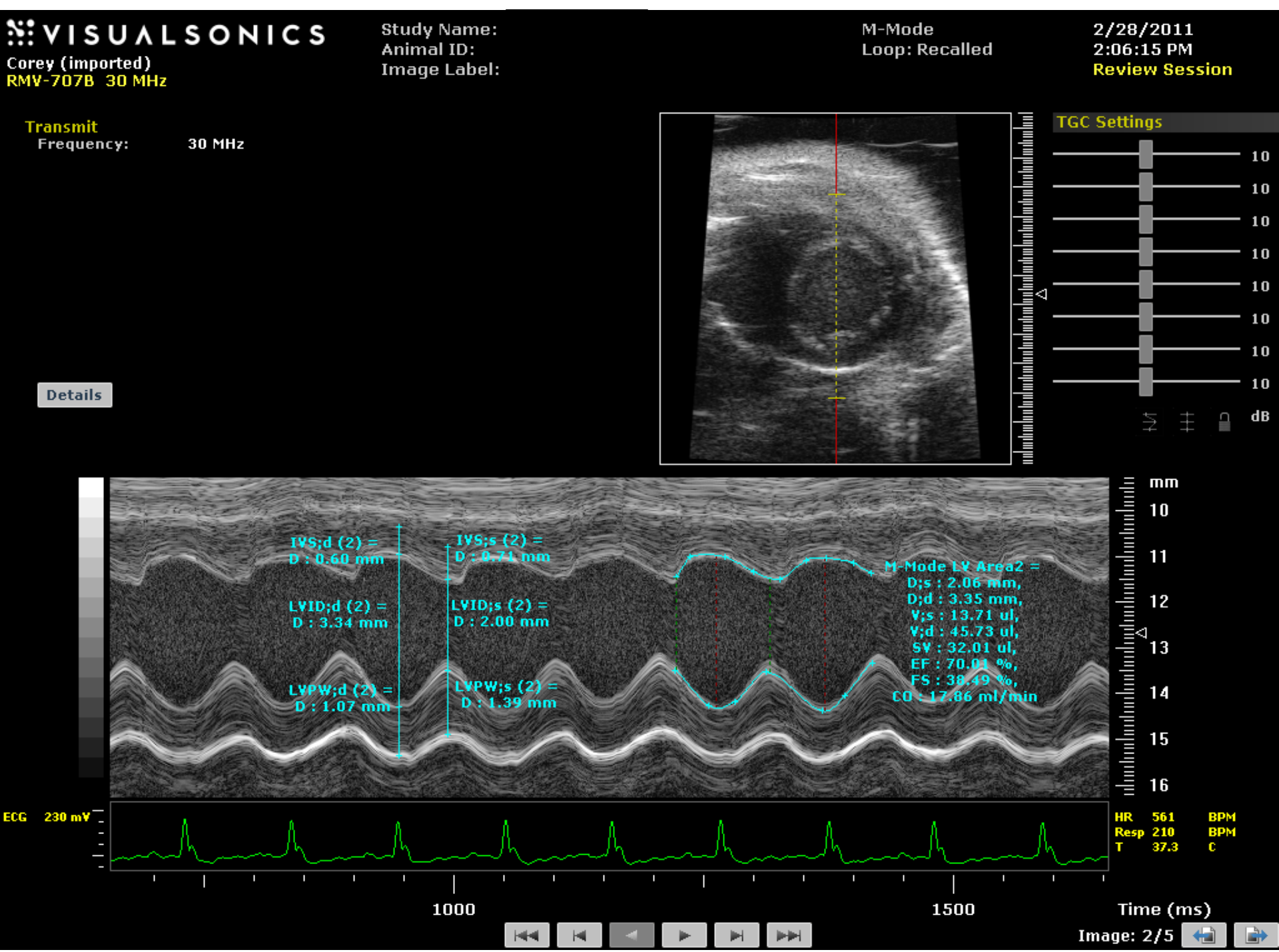
- Roger Price, DVM, Director
- Cobas Mira Chemical chemistry analyzer
- Advia Veterinary Hematology analyzer
- Training platform for Veterinary Pathology
- Necropsies/pathology

Under Development: Cardiac Challenge

Isoproterenol or Dobutamine

- Mimics the effects of exercise (treadmill stress test in humans)
- Stimulates β-adrenergeic receptors →Increase in HR in a healthy animal
- Baseline echo →IP injection of the drug →Post-Injection echo (data points taken every minute for 5 minutes)
- Duration (6-8 min/mouse)

Mouse Phenotyping Core


VISUALSONICS
Corey (imported)
RMV-707B 30 MHz
Study Name:
Animal ID:
Image Label:
M-Mode
Loop: Recalled
2/28/2011
2:06:15 PM
Review Session
Transmit
Frequency: 30 MHz
TGC Settings
10
10
10
10
10
10
10
10
dB
Details
IVS;d (2) =
D : 0.60 mm
IVS;s (2) =
D : 0.71 mm
LVID;d (2) =
D : 3.34 mm
LVID;s (2) =
D : 2.00 mm
LVPW;d (2) =
D : 1.07 mm
LVPW;s (2) =
D : 1.39 mm
M-Mode LV Area2 =
D;s : 2.06 mm,
D;d : 3.35 mm,
V;s : 13.71 ul,
V;d : 45.73 ul,
SV : 32.01 ul,
EF : 70.01 %,
FS : 38.49 %,
CO : 17.86 ml/min
mm
10
11
12
13
14
15
16
ECG 230 mV
HR 561 BPM
Resp 210 BPM
T 37.3 C
1000
1500
Time (ms)
Image: 2/5

Under Development: Respiratory Screen

- Challenge: Methacholine or Ozone
  - assesses airway hyper-responsiveness
- Control Group → Aerosolized isotonic saline

  Experimental Group → Aerosolized Methacholine (mg/ml) of increasing concentrations (4)
- Baseline Collection →Administer drug for 2 min →5 min data collection →10 min rest period →repeat
- Duration (1hr 30min/group of 8 mice)

Mouse Phenotyping Core

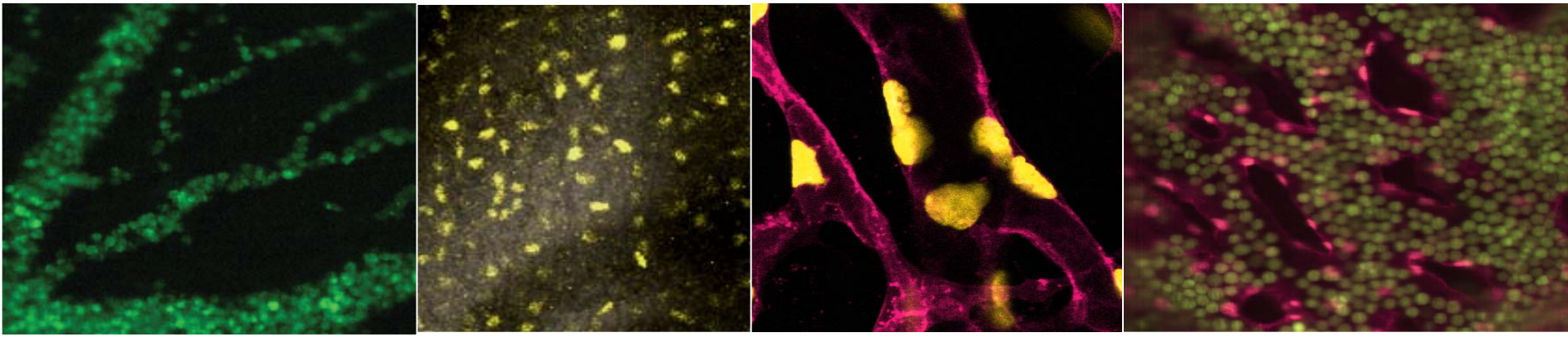
Technology Development
Imaging

Studying embryonic development

Live embryonic imaging is critically important

Confocal microscopy of vital fluorescent markers is a powerful tool

- Barriers: skin/fur, uterine wall



ε-globin-GFP
Flk1-H2B::EYFP
Flk1-myr::mCherry

Mary Dickinson
Irina Larina
Baylor College of Medicine

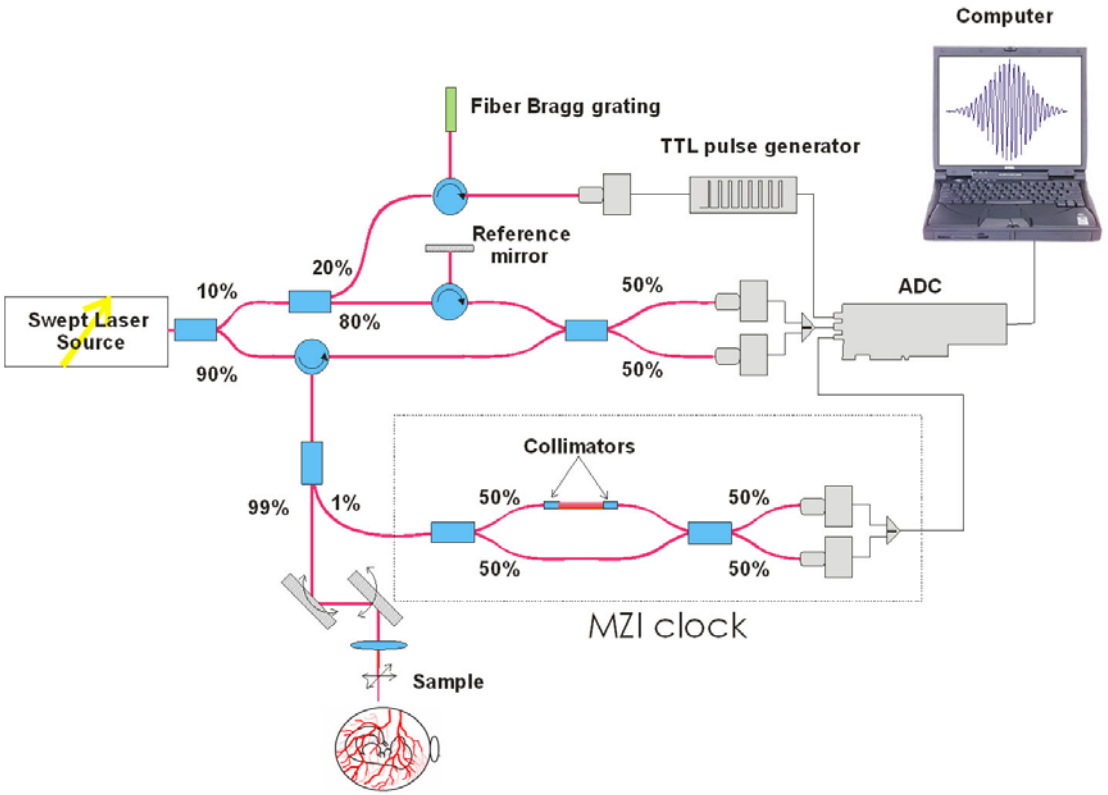
Kirill Larin
University of Houston

Optical Coherence Tomography

Images produced by backscatter from an interferometer
- Uses light instead of sound

Image depth = 2 - 3 mm
- Confocal and 2-photon = 300 - 500 um

High resolution
- 2 - 5 um
- “Image pathology”



Swept-Source OCT system

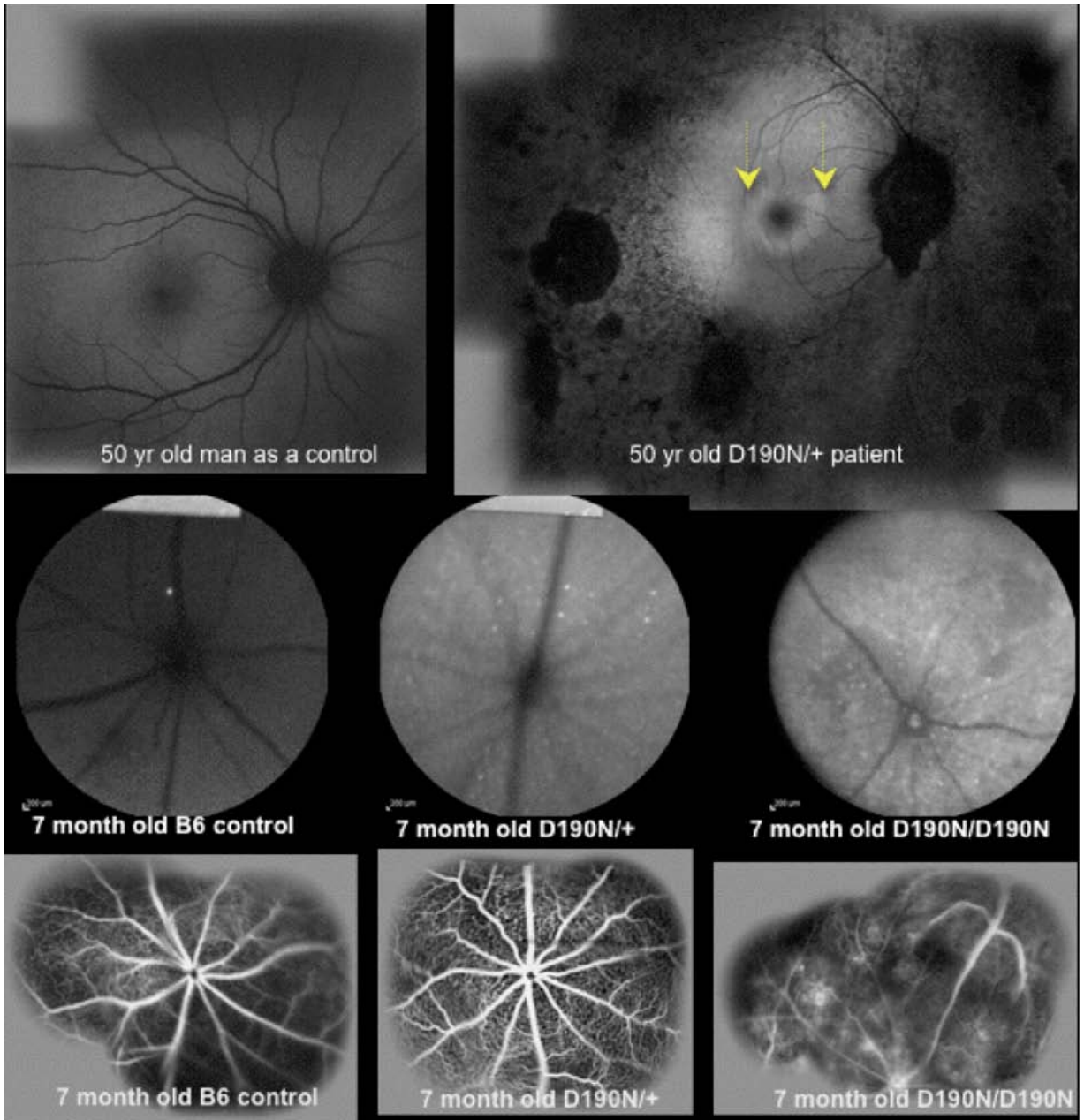
scanning rate - 16 kHz

central wavelength - 1325 nm

spectral width – 100 nm

output power - 12 mW

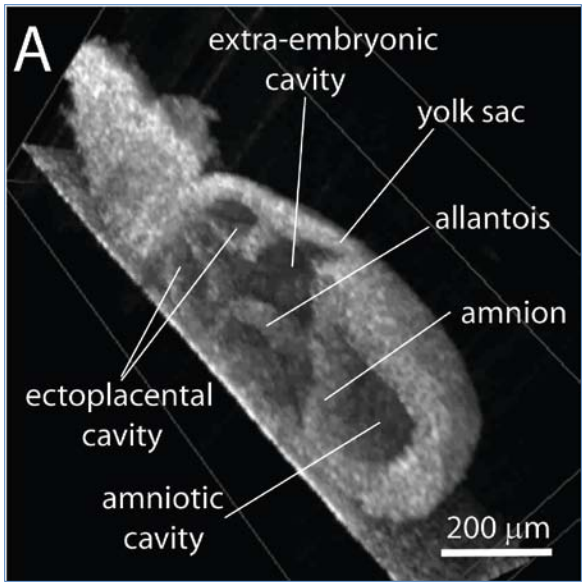
Using OCT to image the adult eye

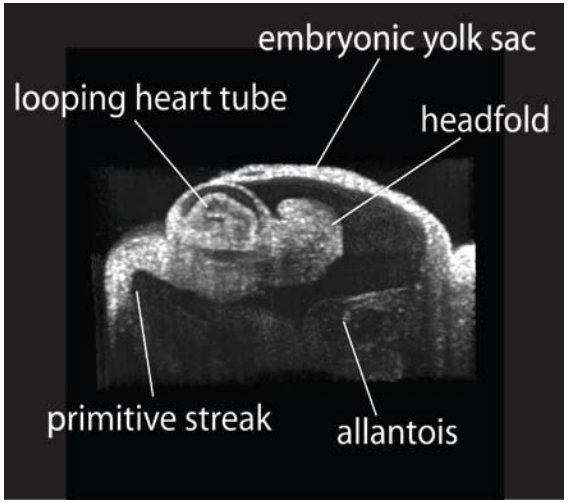


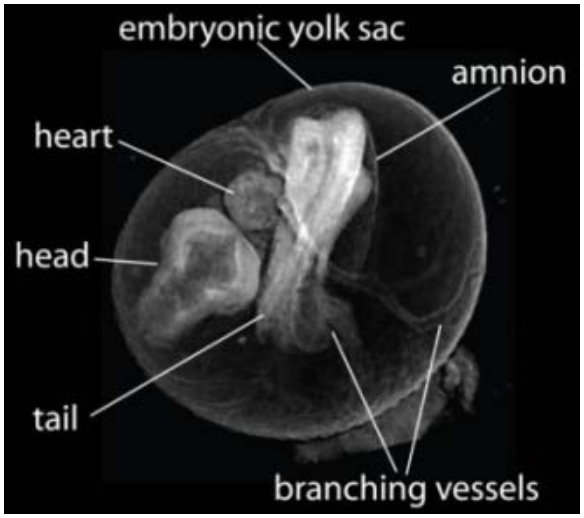
Human:
D190N mutation in RHO causes retinitis pigmentosa: imaged using OCT

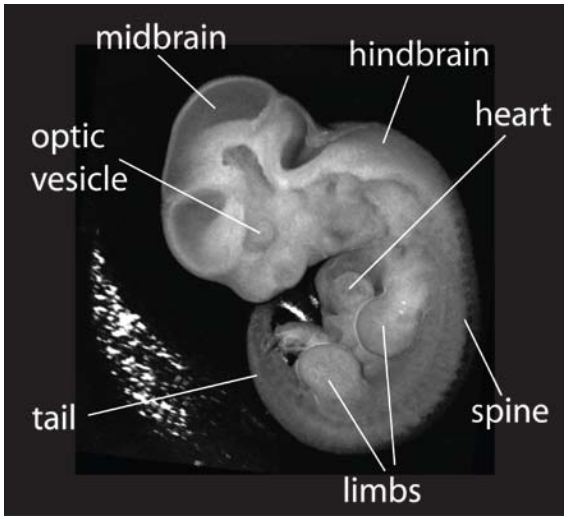
Mouse:
D190N mutation in B6 imaged using OCT

Live OCT imaging of mouse embryos









In utero embryonic imaging

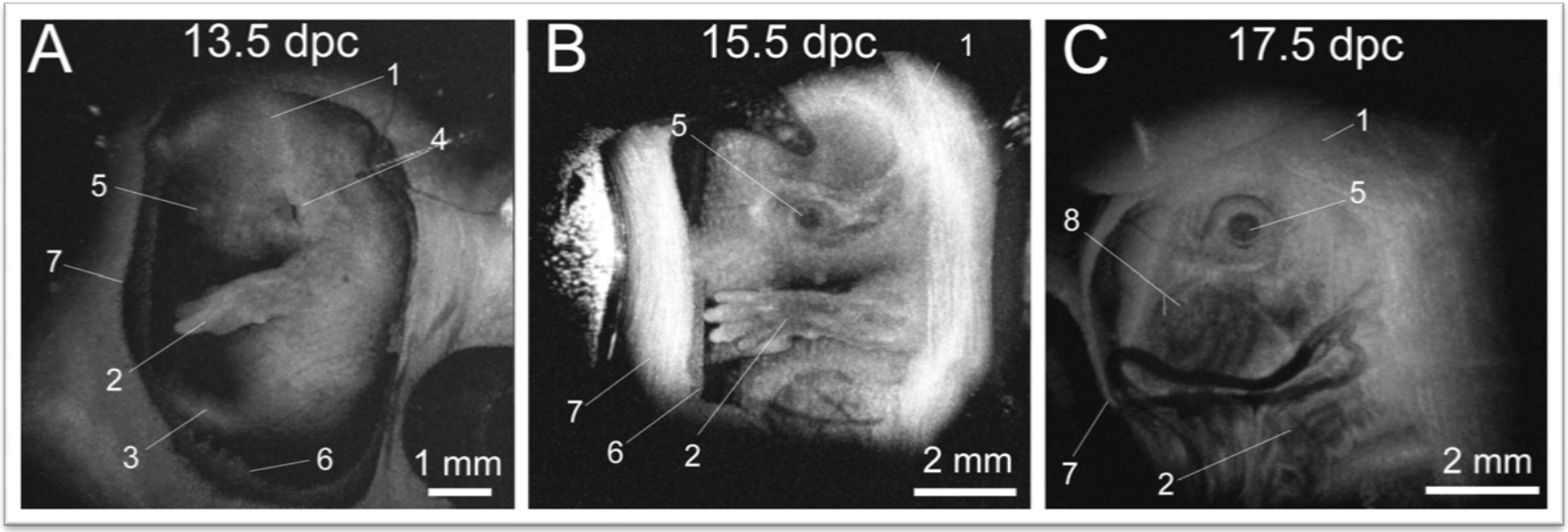
1. Live embryo imaging in sacrificed females (terminal surgeries)

- Pregnant CD-1 females were sacrificed at 12.5 to 18.5 dpc.
- The animal remains on heating pad during the procedure to keep the embryos alive.
- Abdominal wall was cut to expose the uterus and covered with clear plastic wrap to prevent dehydration.
- Live OCT imaging was performed through the uterine wall.

2. Following development in the same embryos (survival surgeries)

- Pregnant females were anesthetized with isoflurane and kept on the heating pad during the whole procedure.
- The uterine horn was exposed for imaging through an abdominal incision.
- After imaging, the incision was stitched back.
- The procedure was repeated after 48 and 96 hours. The animal was sacrificed after the third imaging session.

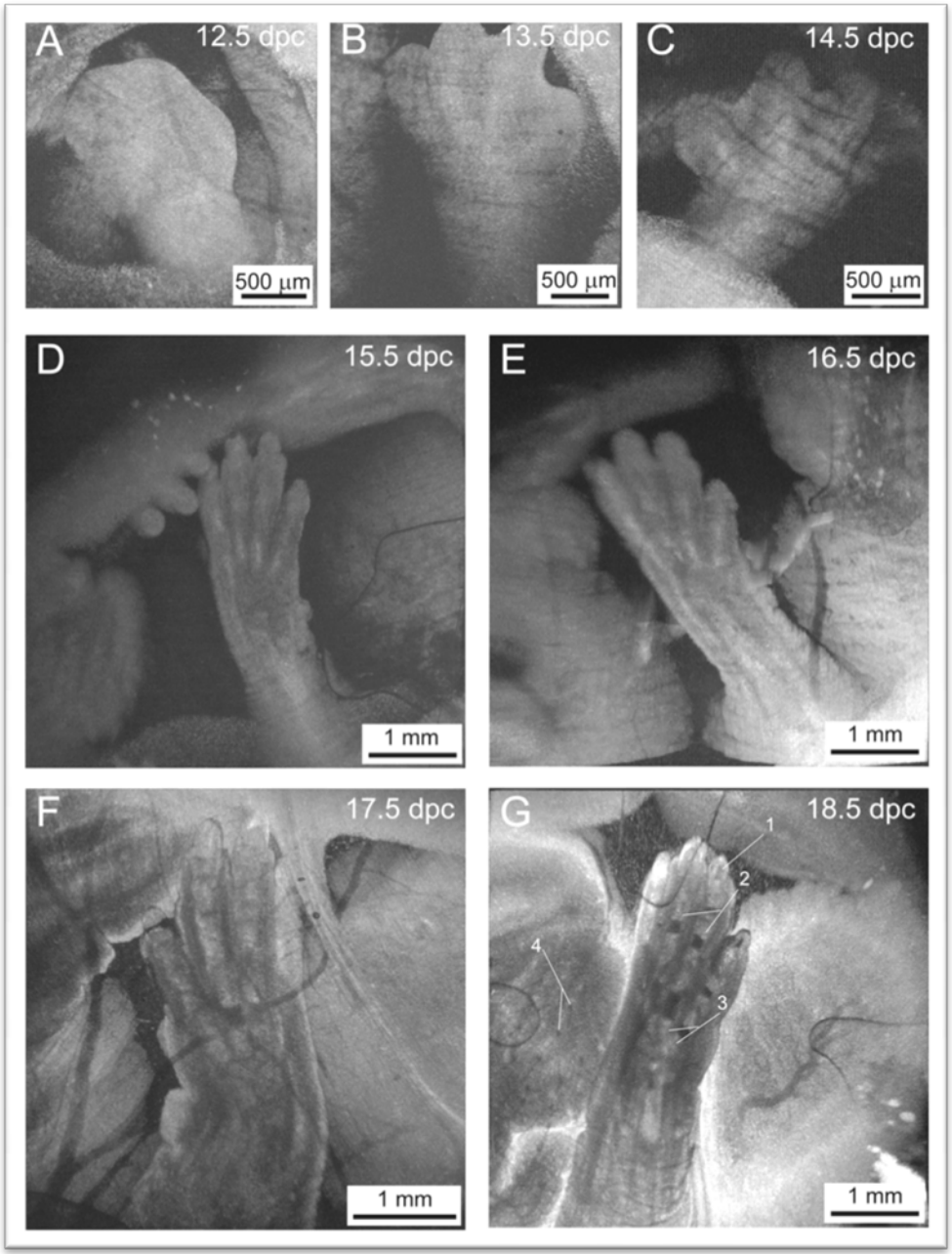
Imaging embryo morphology at different stages of development



1-head
2-forelimb
3-hindlimb
4-pinna of ear

5-eye
6-yolk sac
7-uterine wall
8-follicles of vibrissae

Imaging of embryonic limb development



1-cartilage primordium of distal phalangeal bone

2-cartilage primordium of phalangeal bone

3-cartilage primordium of metacarpal bones

4-follicles of vibrissae

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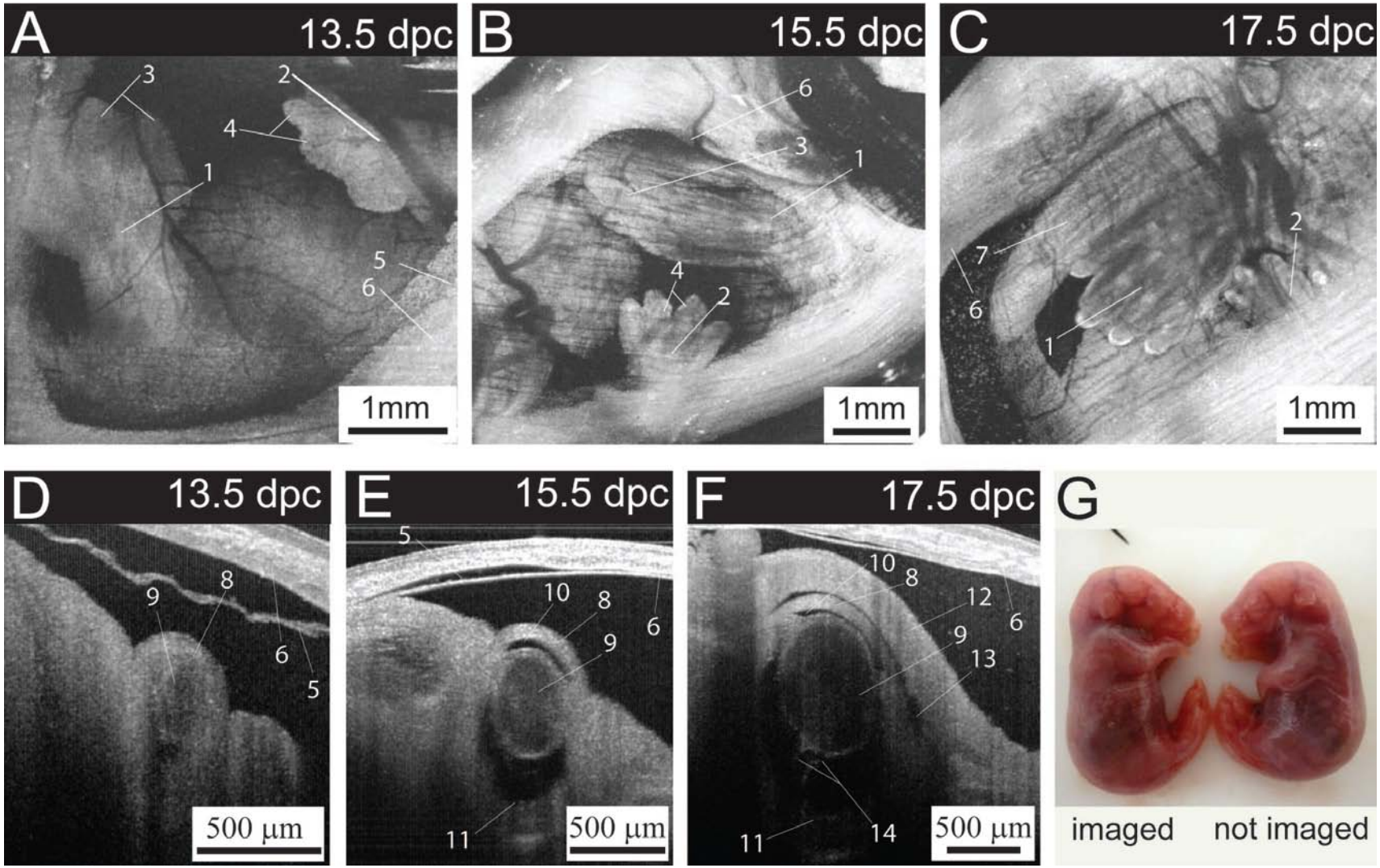
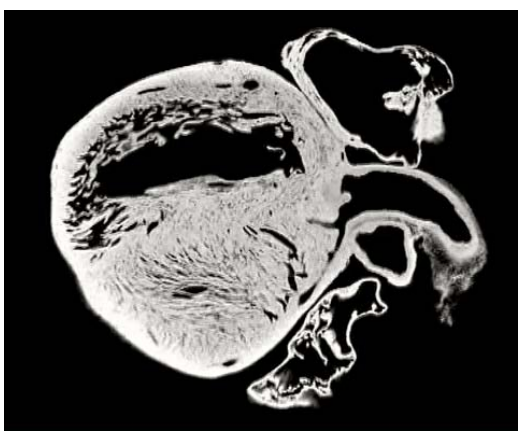
Longitudinal studies

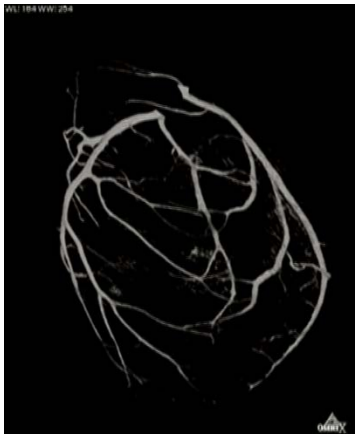


New Technology Evaluation
High-Resolution Episcopic Microscopy (HREM)

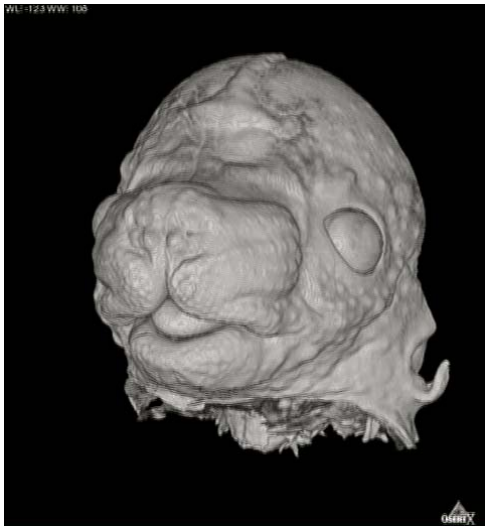
- Shoumo Bhattacharya (Ox), Tim Mohun (NIMR)
- Automated setup
  - Resin embedded samples
  - Fluorescent dyes (acridine + eosin) - stain tissue & provide fluorescent background
  - E8.5 - 17.5
  - Section every 2u
  - Block face serially imaged
  - 24 hours per sample
- Allows resolution to 2 u / voxel
- Can be done after MRI imaging

Weninger et al Nat Genetics 2001; Pieles et al J Anat 2007





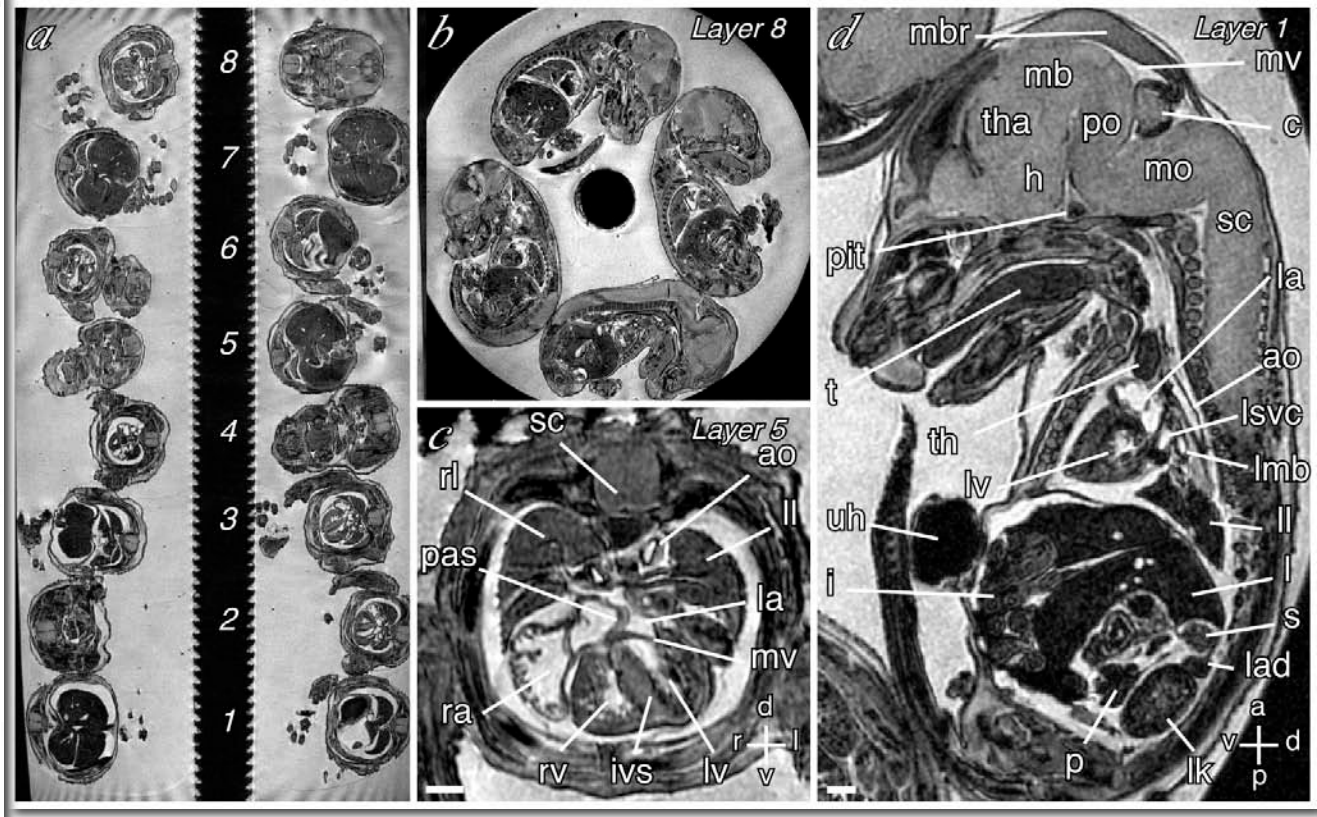
HREM Allows visualisation of coronary vasculature, myocardial anatomy



3D Rendering & Analysis

High Throughput Phenotyping Using MRI

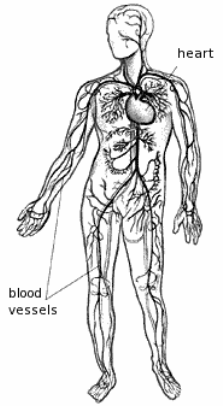
Shoumo Bhattacharya, Jurgen Schneider

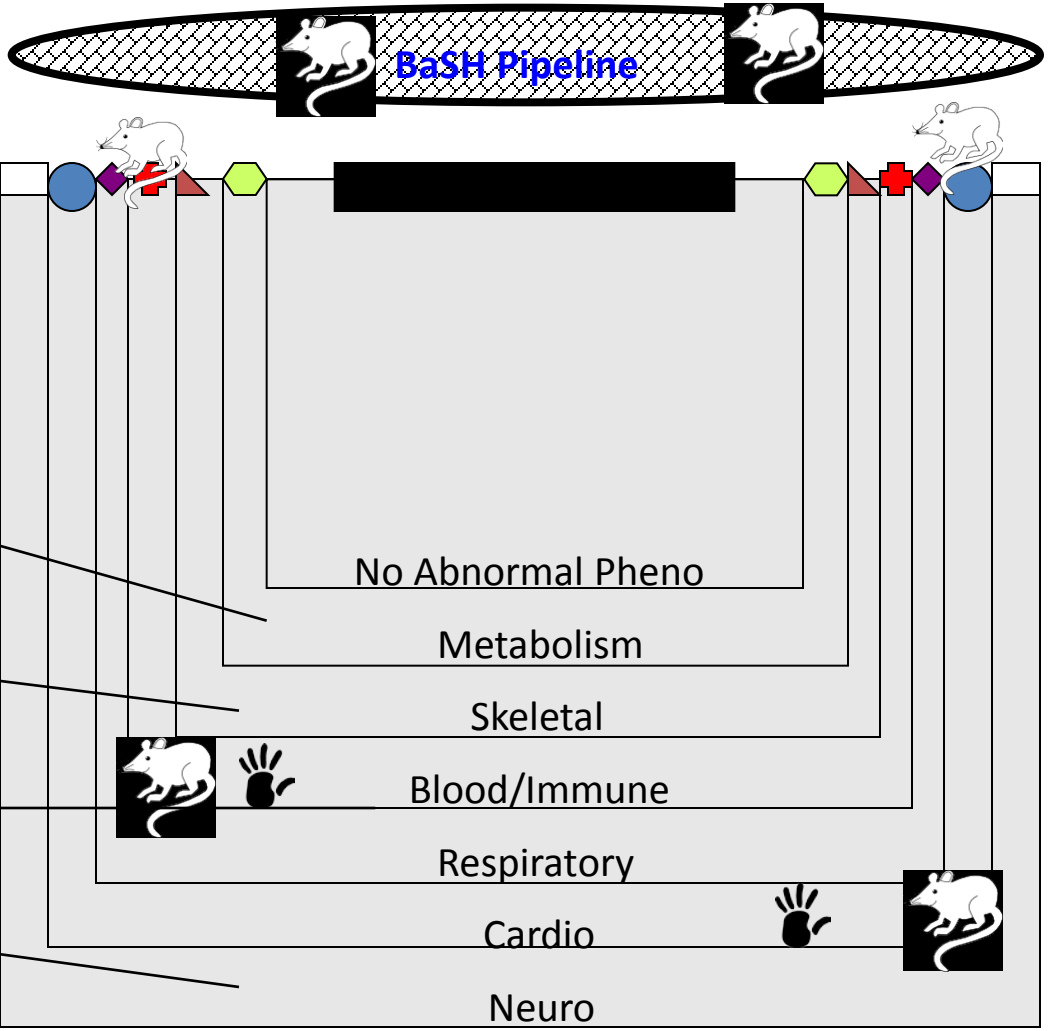


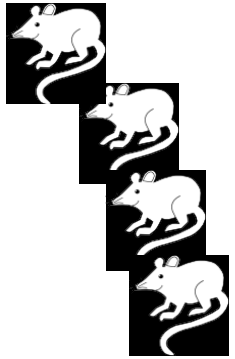
- Magnetic resonance imaging
  - 32 embryos at E15.5 embedded in agarose
  - Imaged overnight on 11.7 T system

Schneider et al BMC Dev Biol 2004

Ultimate Goal:







Diabetes & Endocrinology Research Center

Bone Disorders Program of Texas

Center for Stem Cells & Regenerative Medicine

Neurological Research Institute

Center for Translational Genomics

Providing for the local, national, and international scientific community

Thank You

The BaSH Consortium

BCM: Monica Justice, Rich Paylor, Franco DeMayo, John Sharp, Corey Reynolds

Sanger: Allan Bradley, Bill Skarnes, Ramiro Ramirez-Solis

Harwell: Steve Brown, Tom Weaver

And our collaborators

Mary Dickinson, Kirill Larin, Shuomo Battychara, Tim Mohun, Stephen Wang