Frederick National Laboratory
for Cancer Research

Multiscale Imaging of Tumor Architecture and Dynamics – concept. A strategic direction for the FNL

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DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute
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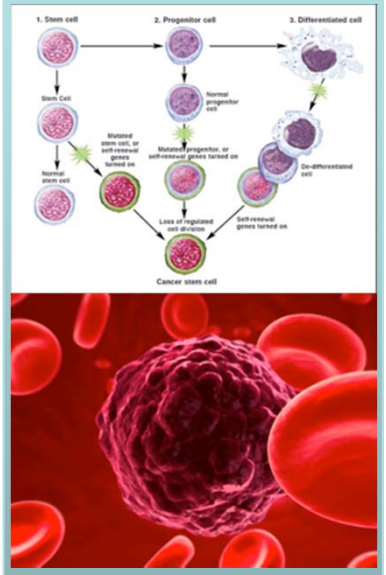
Multiscale Imaging of Tumor Architecture and Dynamics

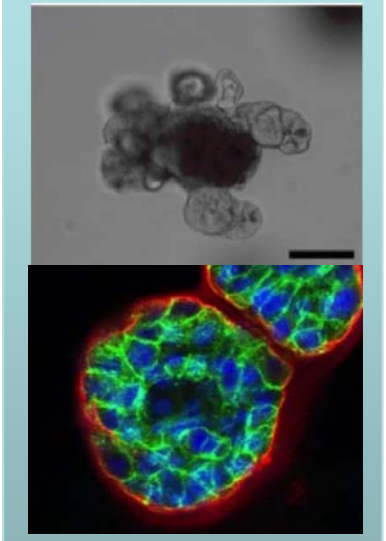
Opportunity, Promise, and Open Questions

Tumor Initiating Cell



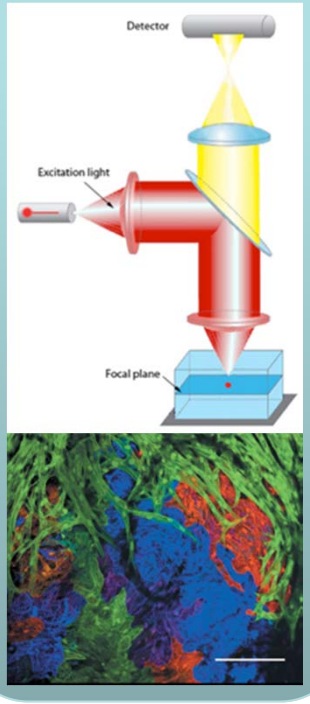
Circulating Tumor Cell

Organoid

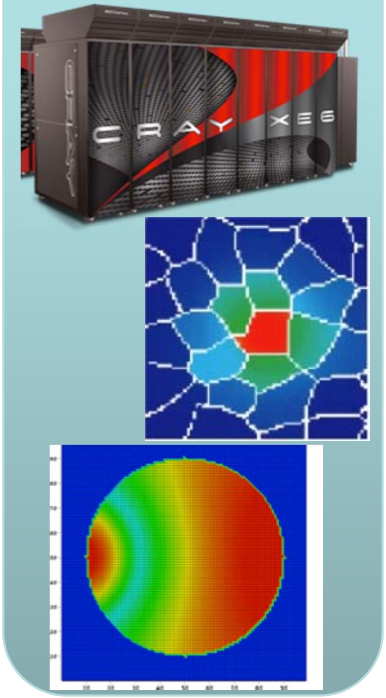


Advances in 3D Culture

Imaging



Computation



Tumorigenic initiation and differentiation programs

Leveraging Frederick National Laboratory: Launch coherent program for a systematic attack on fundamental open questions in tumor dynamics

FNL – Potential to move the needle in NCI defined areas of critical need

NCI Provocative Questions that provoked us!

- PQB – 2 ***What molecular and cellular events in the tumor microenvironment determine if a tumor at the earliest stages of malignant transformation is eliminated, stimulated for further development, or made indolent?***

- PQB – 4 ***What methods can be devised to characterize the functional state of individual cells within a solid tumor?***

- PQC – 4 ***What in vivo imaging methods can be developed to portray the "cytotype" of a tumor — defined as the identity, quantity, and location of each of the different cell types that make up a tumor and its microenvironment?***

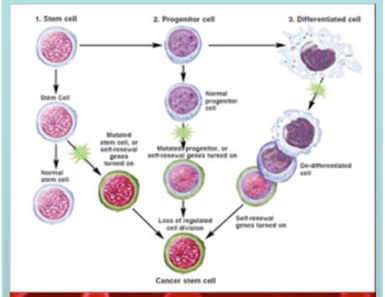
http://provocativequestions.nci.nih.gov/rfa

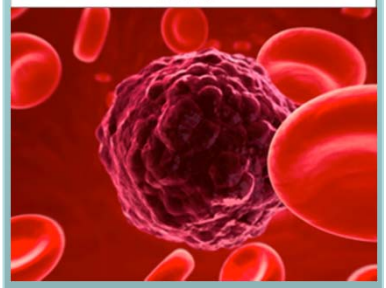
Multiscale Imaging of Tumor Architecture and Dynamics

Opportunity, Promise, and contributing role of emerging 3D models

<= Viable Tumorigenic Progenitors =>

Tumor Initiating Cell

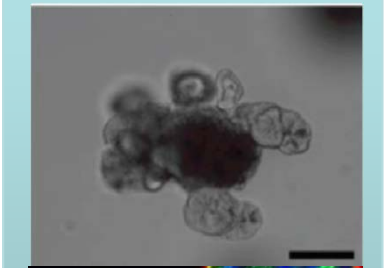


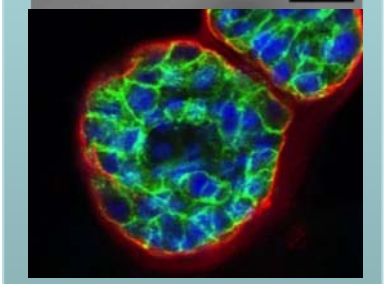


Circulating Tumor Cell

<= Stem Cell and Culture drives fate =>

Organoid





Advances in 3D Culture

Which tumorigenic behaviors in emerging 3D models are relevant to drug resistance and metastatic recurrence? How to Optimize this relevance?

Toward 3D Tumor-Equivalent Models of the Earliest Stages

Tumorigenic initiation and differentiation programs

Seeds for *in vitro* and *in vivo*: Tumor Initiating Cells, Root of Resistance and Metastasis

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- **Need to be able to identify the TICs and expand them**
  - Many current markers are under investigation
  - Significant progress with more physiologically robust examples of architecturally and functionally tantalizing organoids through 3D culture
  - Much more to be done to optimize 3D culture conditions with fundamental tumor biology questions in mind
- **Probe understanding of resistance mechanisms as tumor is de-bulked**
  - TICs are highly tumorigenic and resistant to therapy
  - CTCs are another route to 3D culture models of growing relevance – yet early
  - *Viability and proliferation is not same as metastatic relevance*
- **Potentially the seeds for metastasis**
  - Kills patients, can be idle for many years in the particular niche
  - Seek architectural, molecular, and dynamic characterization of leading edge 3D models with correlative studies *in vivo* by orthotopic implantation to gain TME
  - Human => 3D => mouse; and mouse => 3D => mouse

A strategic direction for the FNL, Framework for a national program

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Launch the FNL program with study of selected 3D model systems and their implantation, as first projects.

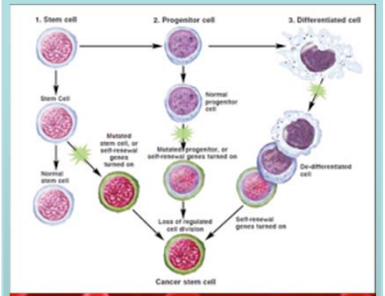
Build and learn, to hone a national lab Multiscale Imaging Engine, including testing and validating high-performance molecular probe toolsets.

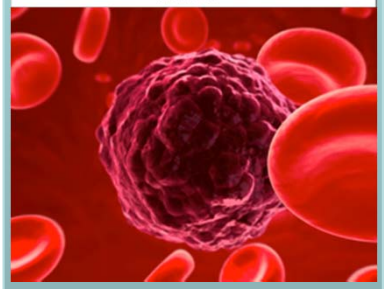
Open the Engine to biologically provocative 3D models and metastatic models in the Community

Multiscale Imaging of Tumor Architecture and Dynamics "MITAD"

Opportunity, Promise, from emerging 3D models

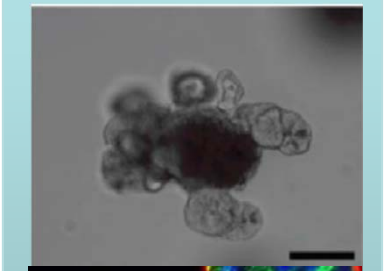
Tumor Initiating Cell

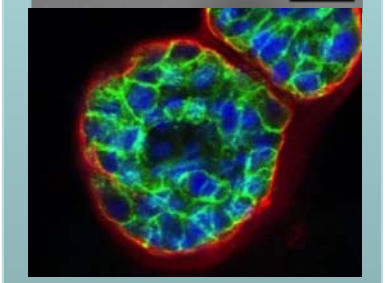




Circulating Tumor Cell

Organoid





Advances in 3D Culture

Tumorigenic initiation and differentiation programs

* **Optimize/enrich/validate imaging probe toolsets as national resource**

in vitro

Molecular profiling, imaging, dynamics in 3D culture & relevance (EMT, drug resistance)

Orthotopic Implantation: tracking determinants of metastatic potential

in vivo

Toward Validation of "series" of Tumor-Equivalent Models of the Earliest Stages

Which tumorigenic behaviors in emerging 3D models are relevant to drug resistance and metastatic recurrence in vitro and in vivo? How to Optimize this relevance?

Framework for a national program at the FNL: three stages

Frederick National Laboratory for Cancer Research

Launch the FNL program with study of selected 3D model systems and their implantation. Build and learn, to hone a national lab Multiscale Imaging Engine, including high-performance molecular probe toolsets. Open the Engine to biologically provocative 3D models in the Community

- **1. Understand the architecture and dynamics of the Clevers' organoid system and/or related state-of-the-art models**
  - Normal stem cell derived, transformed via engineered mutations
  - Patient-derived malignant TICs (Lgr5 marker)
  - Potential inclusion of CTC derived 3D organoid cultures
  - Other *ex-vivo* routes with promise as tumorigenic model systems

Comparative Cytotyping and Dynamics by Molecular Imaging

- **2. Deploy and refine the MultiScale Imaging pipeline for systematic 3D culture, as an engine for comparative functional and architectural analysis of 3D tumor model dynamics and their *in-vivo implantation***
- **3. Open to the community subsequently as a National Lab platform for access and partnership**

MITAD to enable reference platform for leading-edge 3D tumor models

Tumor Initiation

Multicellular Signaling, Architecture and Dynamics

Stromal and Paracrine support. Selection pressures

Immune Response

Tumorigenic

Relevancy

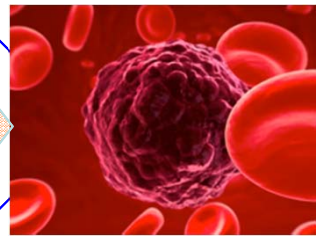
TIC-hu

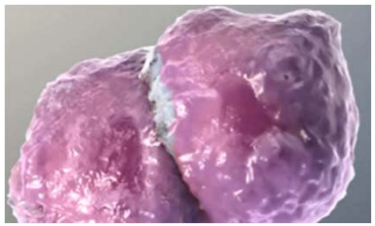
CTC-hu

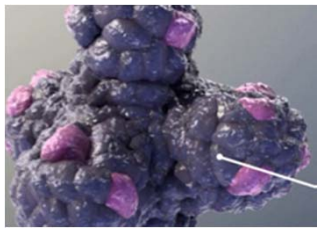
Engineered TIC-hu

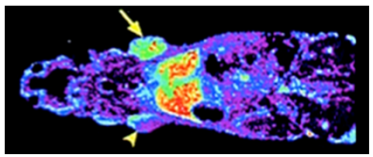
GEMM TIC

Source from community, novel metastatic precursors









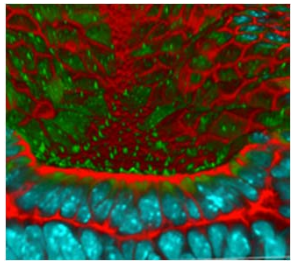
Architectural and Dynamical Complexity

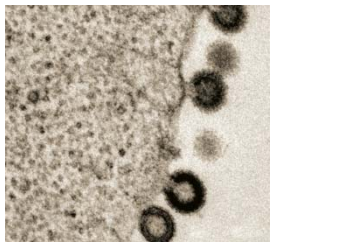
Sources & Culture protocols = Enablers of 3D relevancy

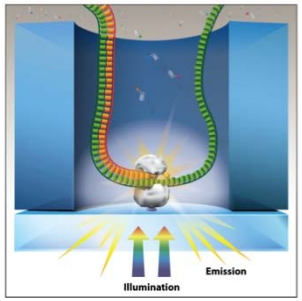
Align, develop this reference lab for candidate 3D models. Advance 3D culture conditions for mechanistic pathway biology, in an engine for comparative functional and architectural analysis.

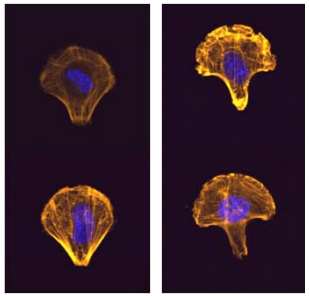
Advance molecular imaging probe toolsets as a national resource

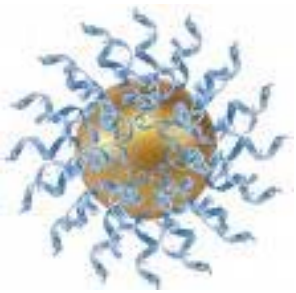
Multiscale Imaging











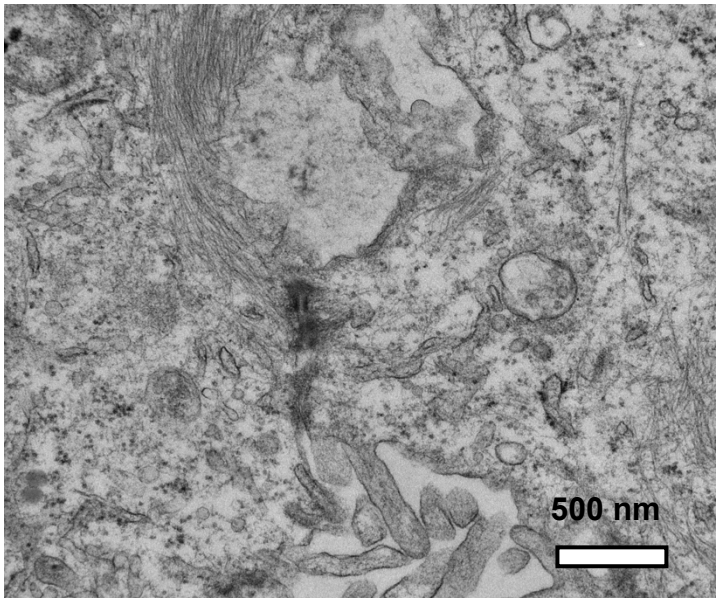
- Subcellular imaging: Molecular Architecture of a single cell
  - EM
  - High Resolution Optical
- Organoids: Cellular Architecture
  - EM
  - Optical
- Animal: Added complexity
  - Optical
  - Ultrasound
  - Nuclear

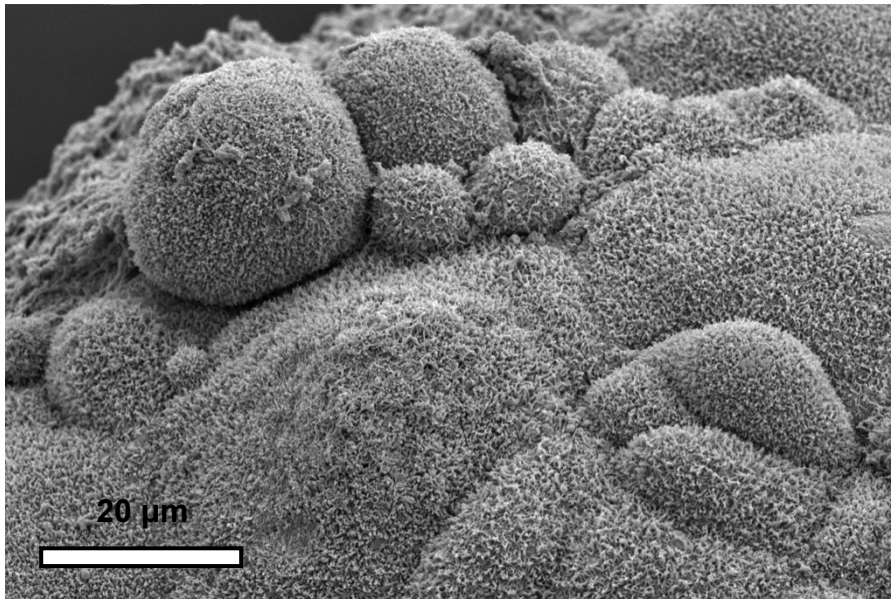
Strategy for Potential Launch

- Leveraging Existing FNL Capabilities
  - Molecular (e.g. Sequencing, Mass Spectrometry)
  - Imaging (EM, Optical, MRI, Ultra Sound, PET)
  - Computation (Image analysis, Data Warehousing, Pathway Modeling)
  - Animal Models (LASP, CAPR)
- Expansion of FNL Capabilities
  - Imaging, Single-Cell sequencing, increased sensitivity
- Partnerships and Collaborations
  - Novel Tumor Equivalent Models and Metastatic Models
  - Imaging Probes (Subcellular -> Clinical)
  - Strengthening existing core areas

EM Ultrastructural Imaging Platform for determination of Cellular architecture

- Traditional thin-sectioning TEM of tissues, cells, and virus pellets
- Negative stain analysis of protein complexes, viruses, nano-particles
- SEM for biological material





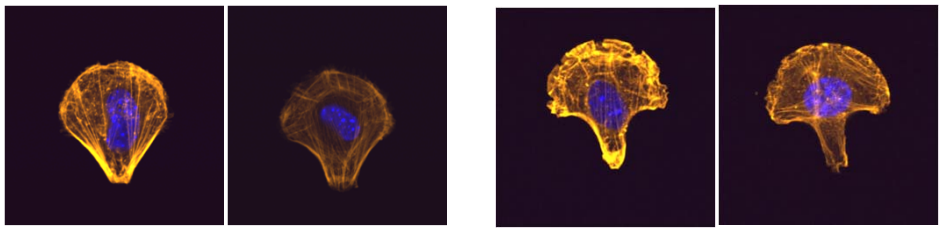
3D Spheroids formed by mesothelioma cells by thin-section EM and by SEM

Optical Imaging: Cellular Architecture and Dynamics

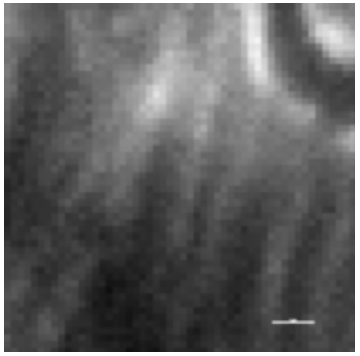
N-STORM super-resolution microscope: Applications include 3D imaging of cellular structures, single molecule imaging.

Image analysis software: Applications include analyzing individual cells in tissues and organoids, molecular colocalization analysis, quantification and modeling of F-actin redistribution in response to drugs.

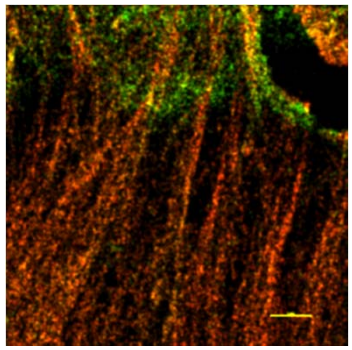
Cell micropatterning technology.



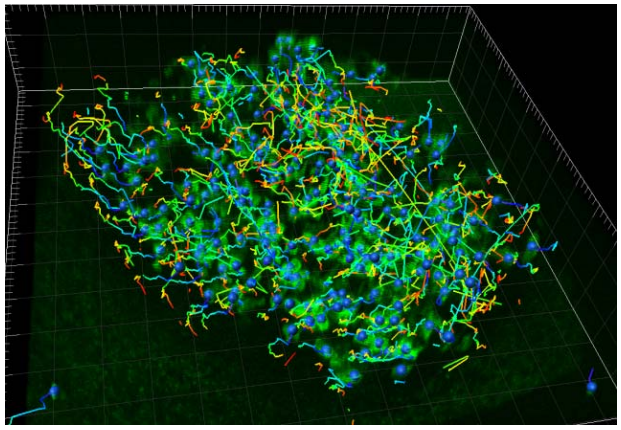
Individual cells grown on fibronectin micropatterns.



F-Actin fibers imaged conventional microscopy



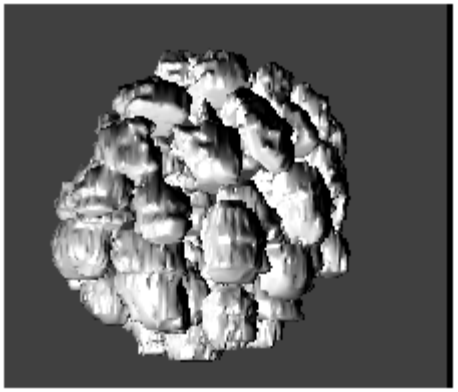
Super-resolution, D-STORM image. (Color represents height)

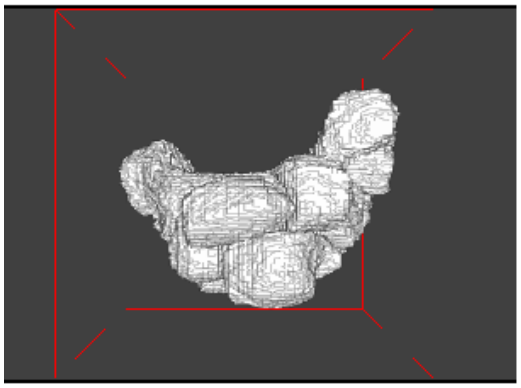


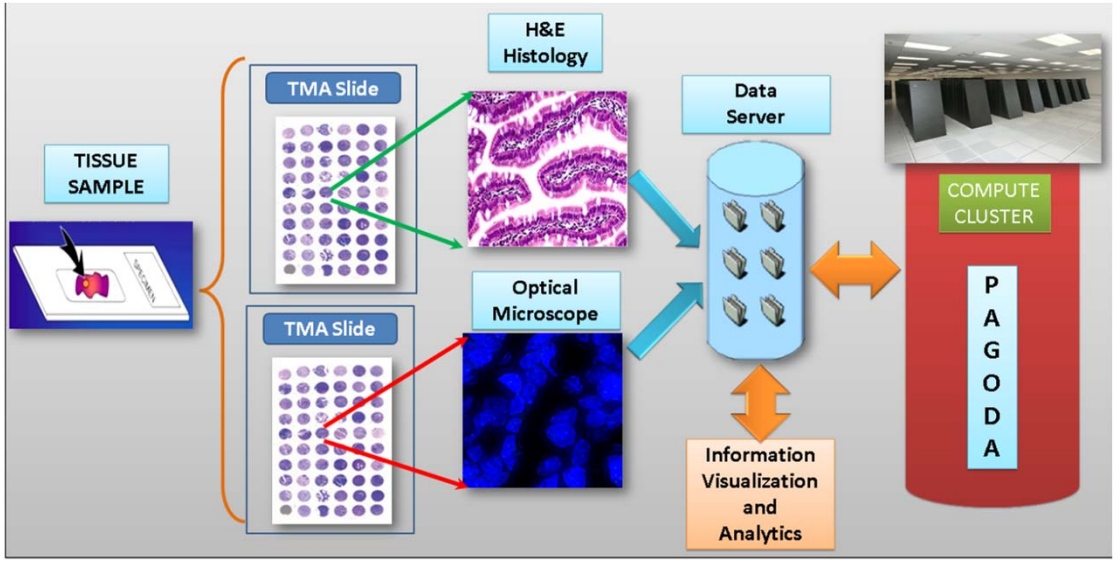
Cell tracking in a live mouse embryo. (In collaboration with Dr. Terry Yamaguchi, CCR)

Integrated tools that can be used to determine the evolution of Cellular Architecture

Segmentation of individual cell nuclei in a fully grown MCF10A acinus (left), and segmentation of individual whole cells in a 3 day old mouse embryo (right)





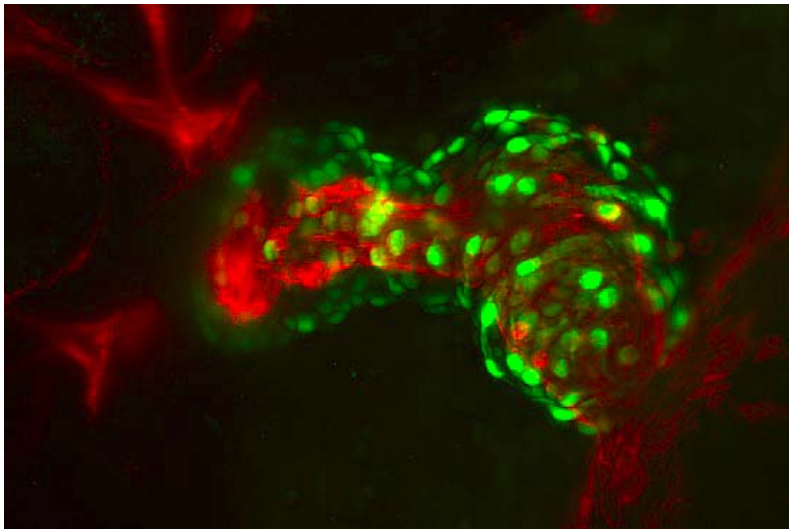


OMAL System Architecture for High Throughput Image Analysis. Currently used for automatic gene positioning in breast cancer detection (In Collaboration with Dr. Tom Misteli, CCR). Visualization developed in collaboration with Dr. Yanling Liu (Imaging and Visualization Group, ABCC)

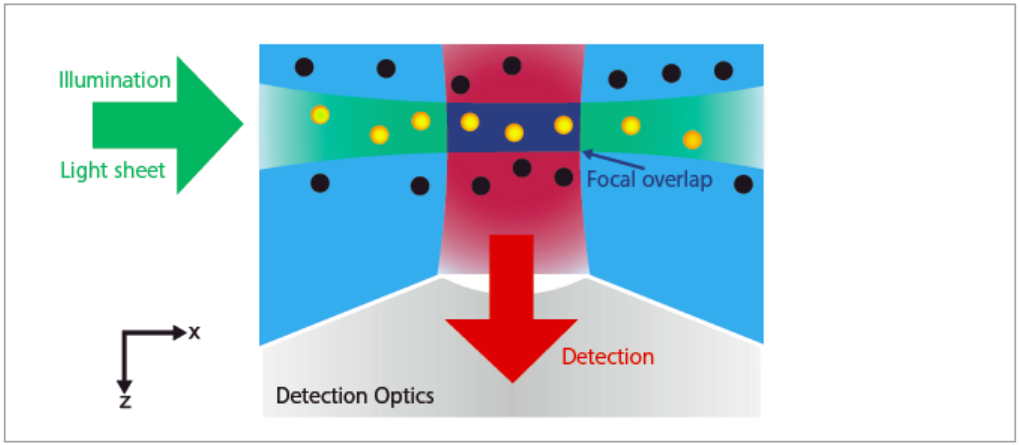
Light-sheet microscopy allows for less invasive analysis enabling the study of dynamics

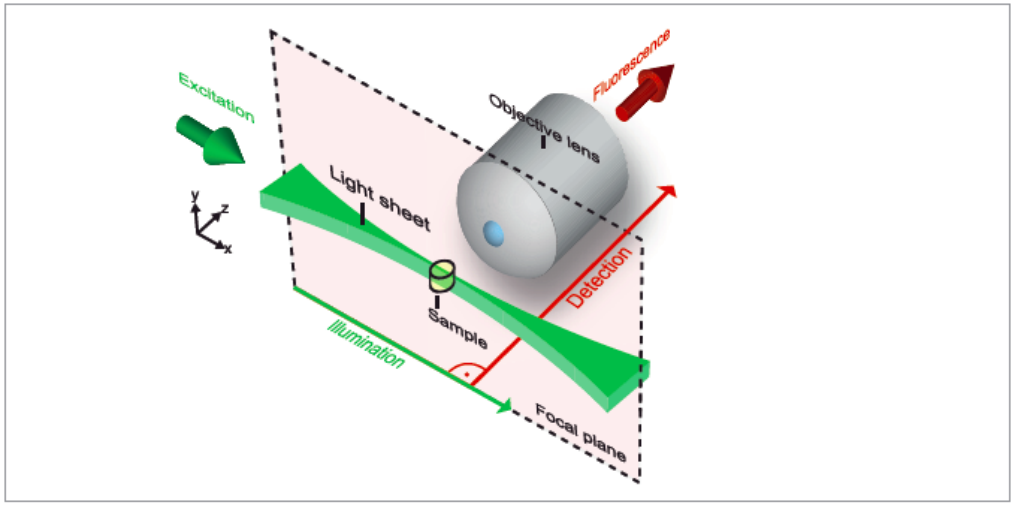
1) Light Sheet Microscopy

2) Fluorescence Life-time Imaging addition to fluorescence correlation spectroscopy



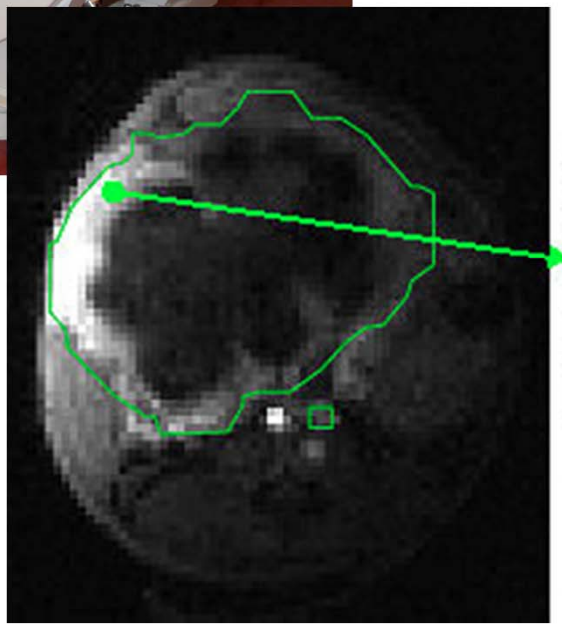
3D rendering of a 2-day old living zebrafish heart. Carl Zeiss Inc.

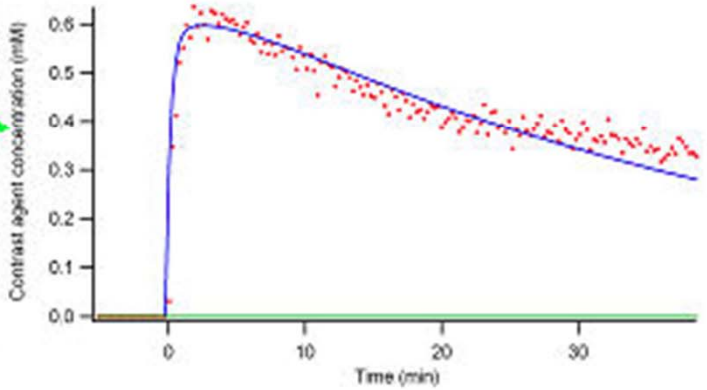




Higher field Instruments for Animal Imaging will provide functional molecular imaging in longitudinal studies

Dynamic contrast-enhanced MRI of orthotopic mouse model of pancreatic cancer. Inset shows time course of contrast agent concentration, which can be fit to models reflecting blood flow and vessel permeability.
http://www.mriresearch.ubc.ca/content/facilities/7T/





FNLCR would be a hub for imaging probes

- There will be a need for optical, magnetic, ultrasound and radiolabeled probes used over all length scales
- There are many groups developing probes in the extramural community, many of which are valuable, but the costs of sharing are too high, and there is limited commercial value
- There is a SIGNIFICANT research value and the FNLCR can be the clearing house for the validated probes (NCL, ACL serve as models)
- For promising probes that can be used in clinical studies to validate the 3D models or for use in diagnostics, can work with partner in moving these studies forward (BDP, NCL, CLIA)

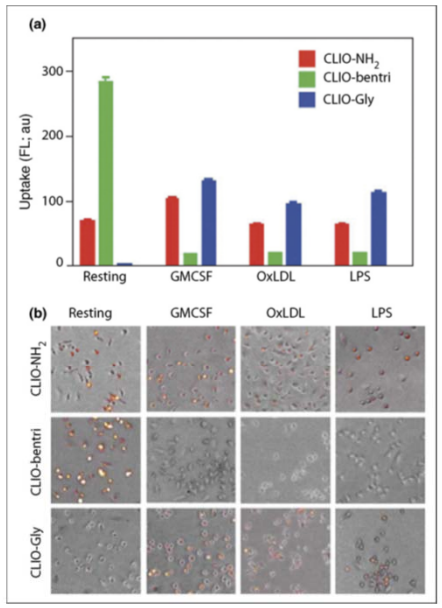
Current Quantitative Analysis at FNLCR for measuring Tumor Architecture & Dynamics

FLUORESCENCE
Cell Tracking
Phenotyping
Tracer Kinetics
Imaging disease-related biomarkers and pathways
HER2
Angiogenesis
Apoptosis
Other probes available
ULTRASOUND
Anatomical volumes
Blood Volume
Blood Flow (Doppler)
Cardiac Function
Tissue Doppler
Image guided injections
Imaging disease-related biomarkers and pathways
Perfusion (Untagged microbubbles
Angiogenesis (VEGFR2 tagged microbubbles)
PHOTO-ACOUSTICS
Vascular Oxygen Saturation
Ischemia

MRI
Dynamic Contrast Enhanced (DCE)-MRI (Permeability)
Dynamic Susceptibility Contrast (DSC)-MRI (Perfusion)
Tracer Kinetics
Anatomical volumes
NUCLEAR
Imaging disease-related biomarkers and pathways
[^{18}F]FDG: Glucose Metabolism
[^{18}F]FLT: Proliferation
HER2 assay: [^{111}In] and [^{89}Zr] labeled Trastuzumab
HER1 assay: [^{111}In] and [^{89}Zr] labeled Panitumumab
Other probes available
Tracer Kinetics
Biodistribution
Internal Radiation Dosimetry
BIOLUMINESCENCE
Cell Tracking
Tumor Growth
Metastasis

There are many probes that can be used to identify cells & measure changes in metabolism and RNA expression

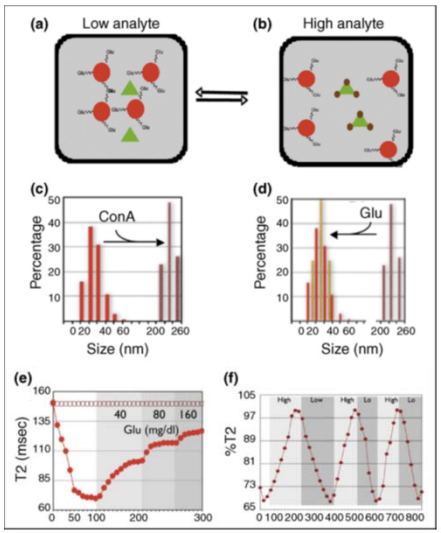
Figure 2



Generation of an iron oxide nanoparticle library for macrophage sensing. The parent compound CLIO-NH_2 is taken up by both resting and activated macrophages. Activation of the macrophages by granulocyte macrophage colony stimulating factor (GMCSF), oxidized LDL (OxLDL) or lipopolysaccharide (LPS) produces a significant increase in avidity for the compound CLIO-Gly, which is otherwise minimally taken up by resting macrophages. Conversely, the agent CLIO-bentri is taken up by resting macrophages, but not by activated macrophages. **(a)** Bar chart showing the degree of uptake of the different iron oxide nanoparticles. The *y* axis shows the fluorescence on uptake in arbitrary units (au). **(b)** Near-infrared fluorescence microscopy of probe uptake (color scale) fused with phase contrast images of the macrophage populations. (Figure adapted from [28**] with permission.)

Weissleder R, Kelly K, Sun EY, Shtatland T, Josephson L: Nat Biotechnol 2005, 23:1418-1423.

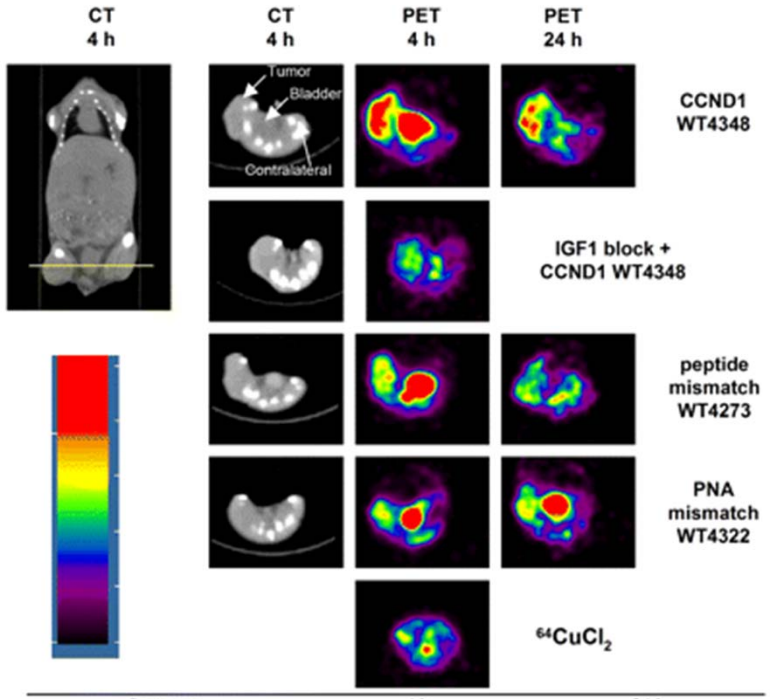
Figure 3



Magnetic nanosensing of glucose concentrations. **(a)** In the presence of low glucose concentrations the CLIO-glucose nanoparticles (red) bind to concavalin-A (green) and form clusters. **(b)** In the presence of high glucose (brown) concentrations, CLIO-glucose is displaced from concavalin-A causing the disassembly of the nanoswitch. **(c,d)** Size distribution of nanoparticles by light scattering. At baseline (red) the majority of nanoparticles are between 20–40 nm in size, increase to 200–260 nm after the addition of concavalin-A (brown) and then return to their baseline size distribution (yellow) after the addition of glucose to the solution. **(e,f)** Changes in glucose concentration can be sensed by detecting changes in the transverse relaxation time (T2), induced by assembly or disassembly of the nanoswitch. (Figure adapted from [34**] with permission.)

membrane is inadequate. The use of magnetic relaxation switches to image local enzyme activity, however, is com-

Sun EY, Weissleder R, Josephson L: Small 2006, 2:1144-1177.

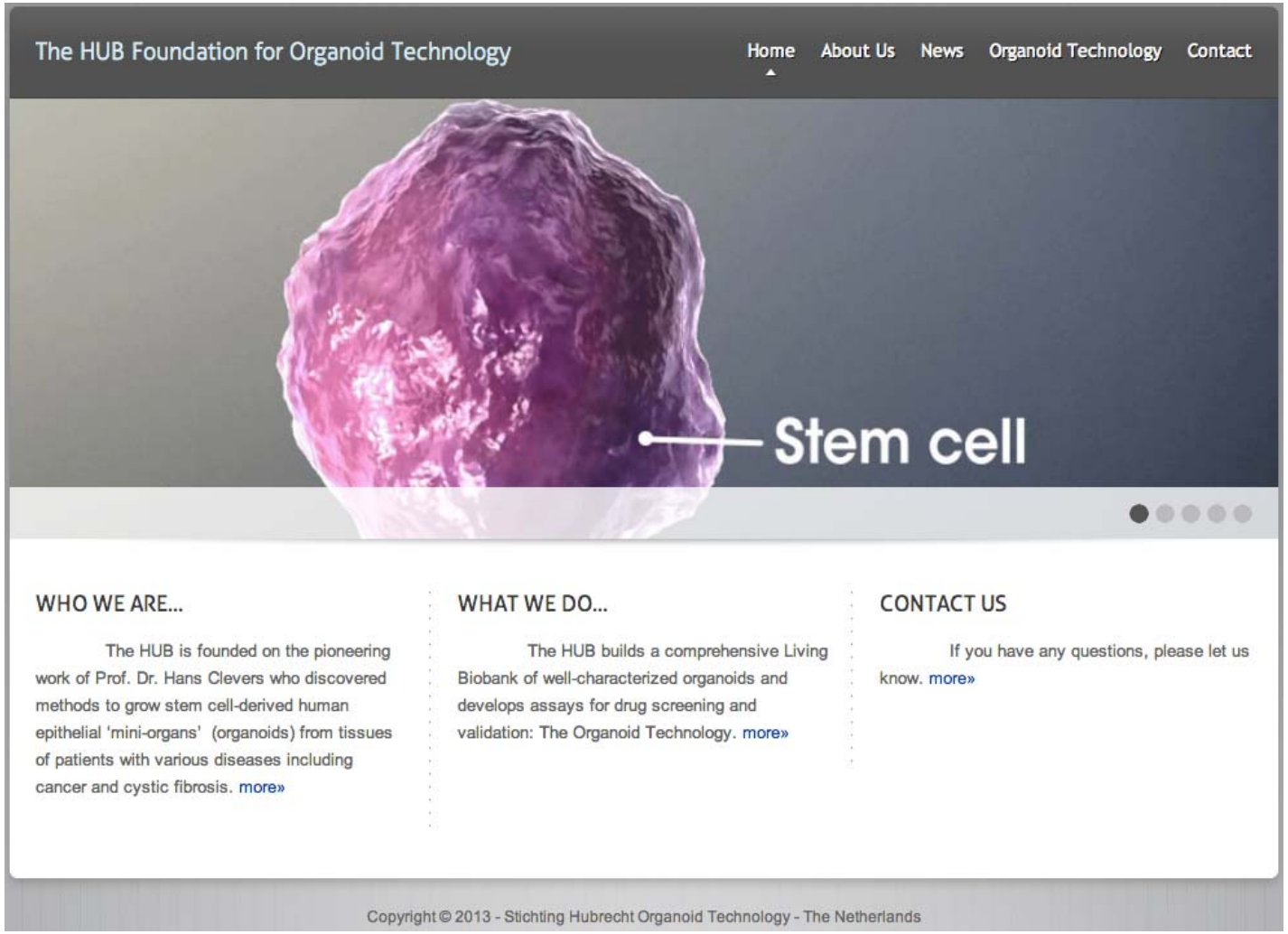


Sequence	4 h	24 h
CCND1 probe [^{64}Cu]WT4348	6.83 ± 3.41 (n=5)	7.87 ± 1.99 (n=4)
IGF1+[^{64}Cu]WT4348	3.66 ± 0.17* (n=2)	not done
peptide mismatch [^{64}Cu]WT4273	3.82 ± 1.06* (n=5)	5.04 ± 0.67* (n=4)
PNA mismatch [^{64}Cu]WT4322	3.37 ± 1.43* (n=4)	4.53 ± 1.77* (n=4)
free $^{64}CuCl_2$	2.97 (n=1)	not done

*significantly different ($p<0.05$) based on Holm-Sidak all pairwise multiple comparison

Transverse microPET and microCT images of immunocompromised NCr mice bearing human MCF7 ER+ xenografts. Tian X, Aruva MR, Zhang K, Shanthly N, Cardi CA, Thakur ML, Wickstrom E. J Nucl Med. 2007 Oct;48(10):1699-707.

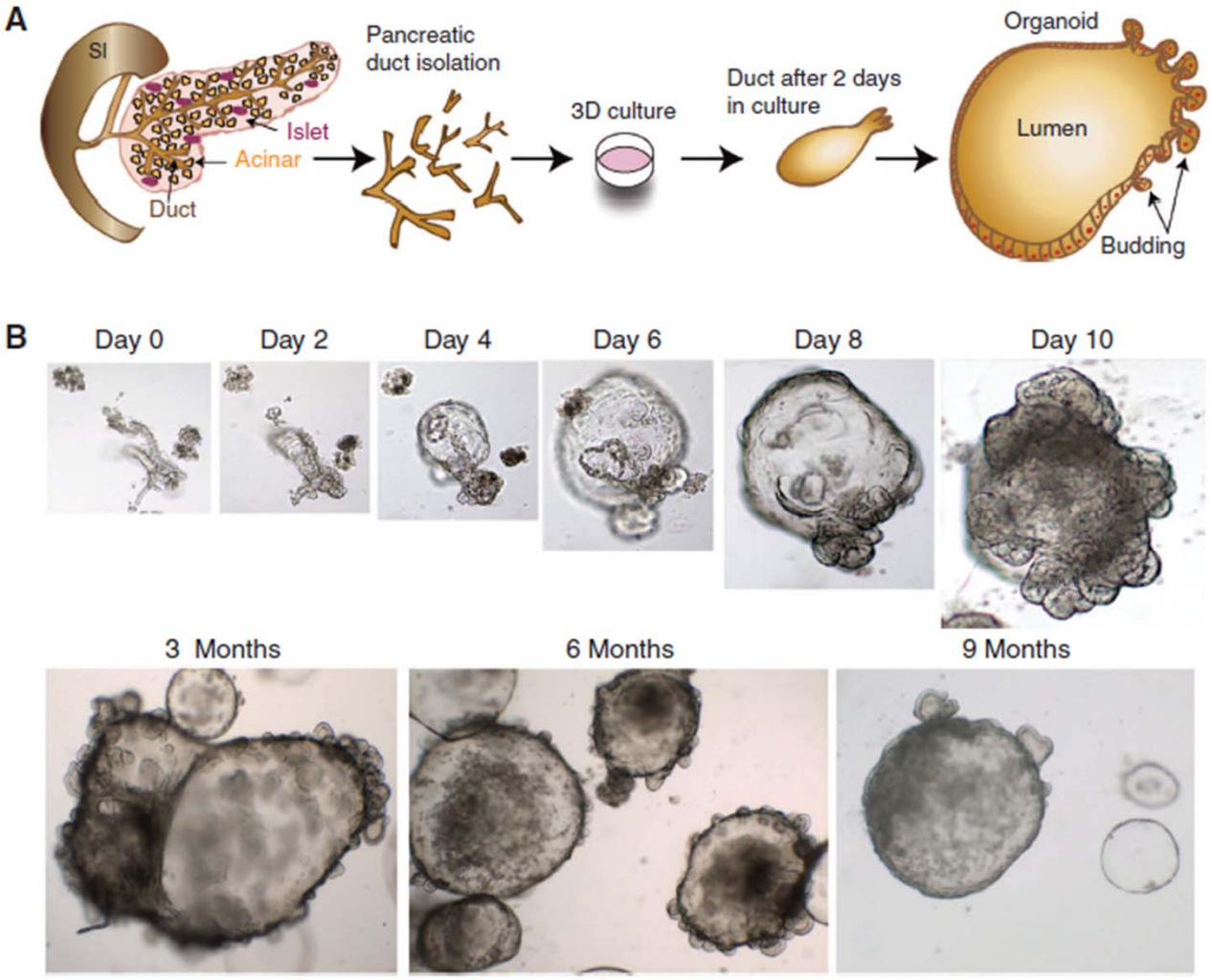
Multiscale Application – Potential collaboration with The HUB Foundation for Organoid Technology’



Multiscale Scenario: Tumorigenic and Tumor Dynamics

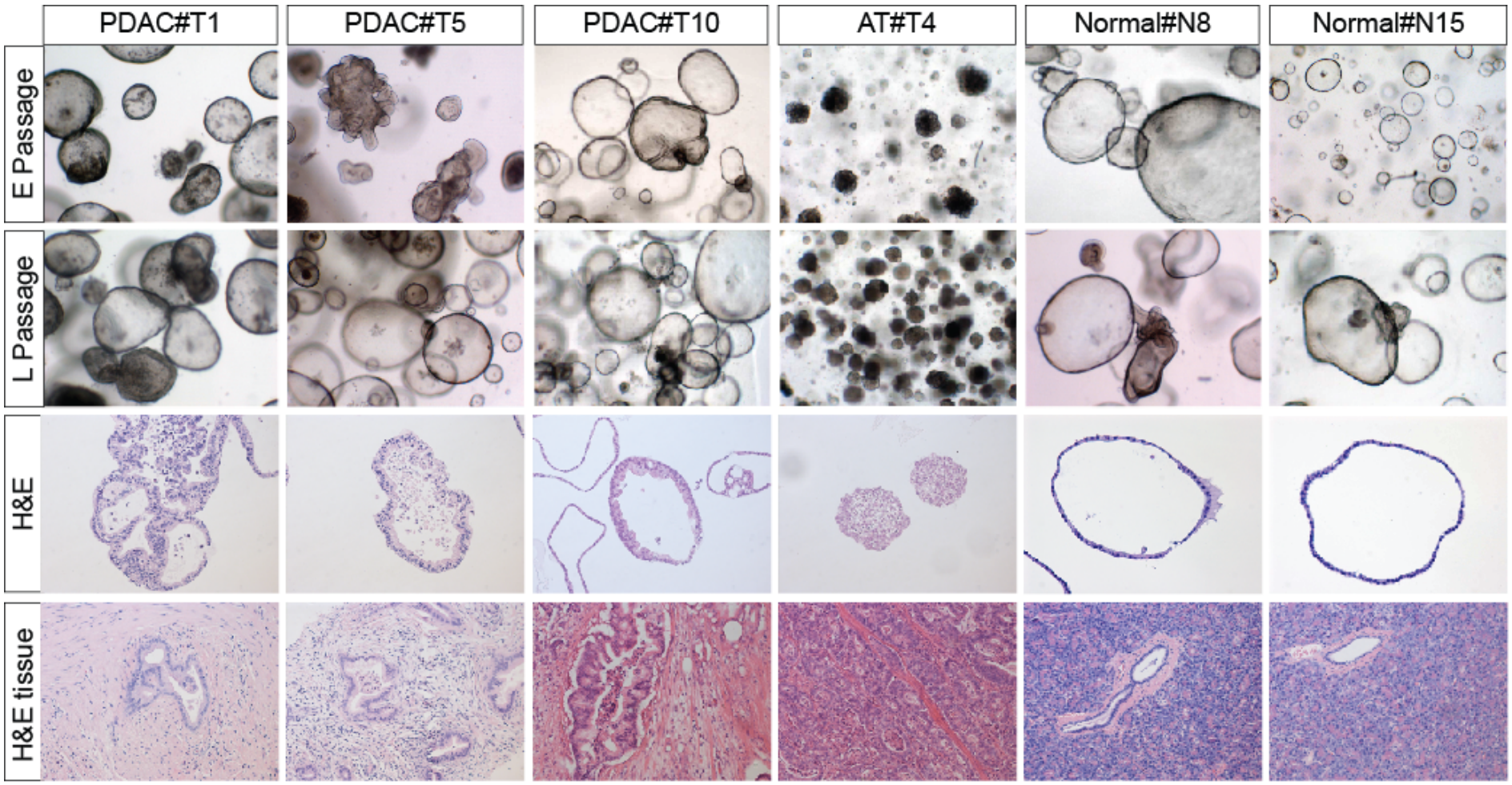
- Potential Collaboration of FNL with Clevers' Organoid Hub
- Other Innovative sources can join in first wave of projects into Engine
- Organoid model scenario:
  - Earliest Stage in Tumor Dev: Response to external stimuli
  - Molecular profiling within architecture: Genomic, Expression (RNAseq), Metabolites, Cell-surface (FACS), super-resolution optical
  - Determine molecular/cellular features that may be correlated with metastatic potential *in vitro* and *in vivo* (molecular probes & imaging)
  - Monitor dynamics of organoid as it changes from *in vitro* to *in vivo* upon orthotopic implantation, of tumor and of stromal and immune cells
- Reference organoid system has been validated as TIC/engineered, and TIC-PDAC, TIC-CRC formats, and with reference behavior of organoids arising from culture of normal stem cells

Establishment of Pancreas Organoids from normal Pancreatic Ducts or Cancers



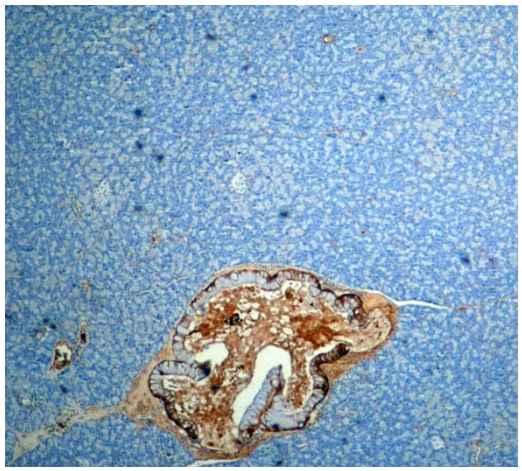
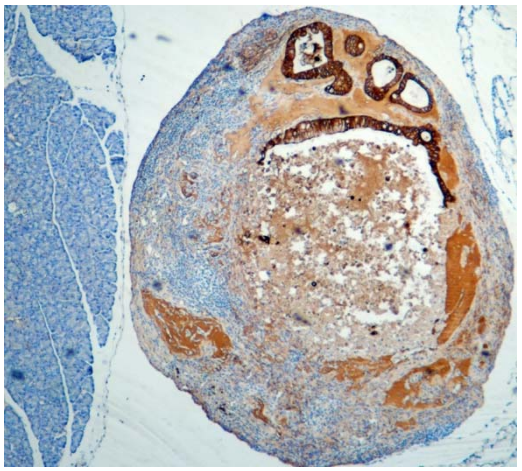
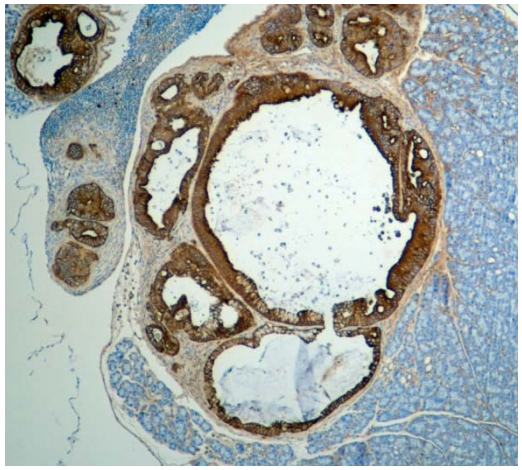
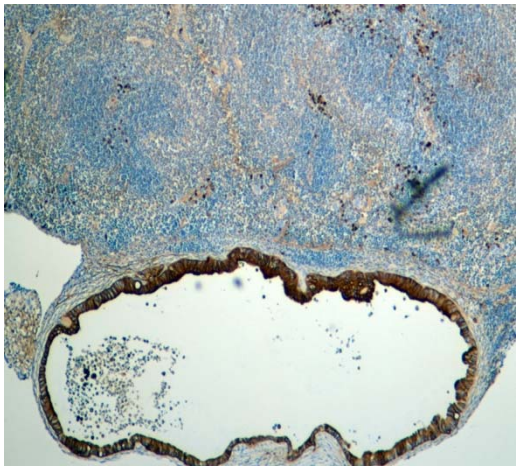
Work presented in this slide kindly provided by Prof. Hans Clevers, Hubrecht Institute, Netherlands

Human Pancreas Organoids mimic the original tissue - demonstrating the importance of measuring Architecture



Work presented in this slide kindly provided by Prof. Hans Clevers, Hubrecht Institute, Netherlands

Orthotopic xenografts of T1 and T5 samples metastasize to distant sites

	Pancreas	Lymph node
PDAC#T1		
	Pancreas	**Spleen**
PDAC#T5		

CAM5.2
Human specific ab

Work presented in this slide kindly provided by Prof. Hans Clevers, Hubrecht Institute, Netherlands

A strategic direction for the FNL, Framework for a national program

Frederick National Laboratory for Cancer Research

Launch the FNL program with study of selected 3D model systems and their implantation, as first projects.

Build and learn, to hone a national lab Multiscale Imaging Engine, including testing and validating high-performance molecular probe toolsets.

Open the Engine to biologically provocative 3D models and metastatic models in the Community

FNLCR today and tomorrow

- Large-scale team science; Systematic reproducible protocols for 3D culture and molecular imaging; Expansion of probe toolkit for *in vitro* and *in vivo* mechanistic imaging
- MITAD as Engine for comparative functional and architectural analysis of candidate 3D tumor models and their dynamics
- Field of Opportunity: advances in 3D culture of TIC-hu (tumor/ engineered), CTC-hu, TIC-mouse, chip-based tissue systems…

MITAD enables future comparative characterization projects:
Access for the National Cancer R&D Community, NCI intramural, extramural, academic, pharma, non-profit

Envisage strategic technology partnerships on leading-edge instrumentation and molecular *in vitro* / *in vivo* imaging probe technology, and entrepreneurial activity linked to the FNL hub

MITAD to enable reference platform for leading-edge 3D tumor models

Tumor Initiation

Multicellular Signaling, Architecture and Dynamics

Stromal and Paracrine support. Selection pressures

Immune Response

Tumorigenic

Relevancy

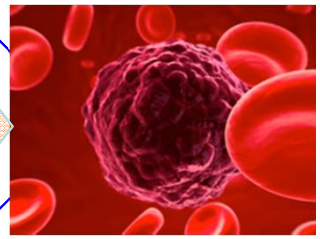
TIC-hu

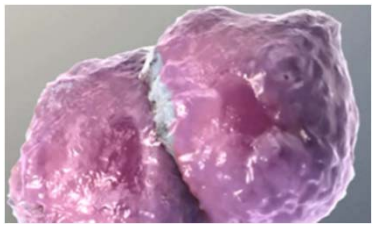
CTC-hu

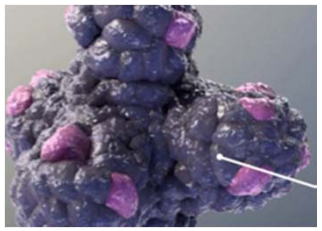
Engineered TIC-hu

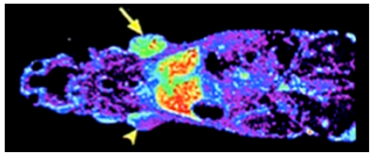
GEMM TIC

Source from community, novel metastatic precursors









Architectural and Dynamical Complexity

Sources & Culture protocols = Enablers of 3D relevancy

Align, develop this reference lab for candidate 3D models. Advance 3D culture conditions for mechanistic pathway biology, in an engine for comparative functional and architectural analysis.

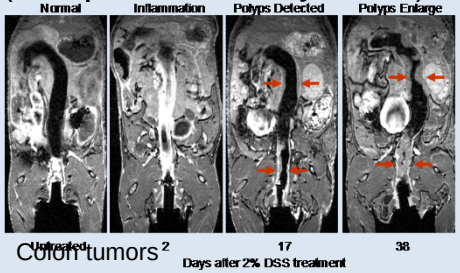
Advance molecular imaging probe toolsets as a national resource

- extras

Current SAIP Instrumentation for Multiscale Imaging

3T MRI

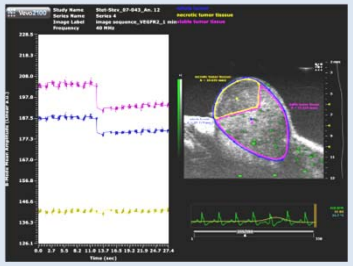
(Philips Medical Systems)



Colon tumors

ULTRASOUND

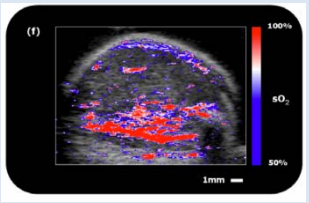
(Vevo 2100; VisualSonics, Inc.)



Imaging angiogenesis using microbubbles tagged with VEGFR2 antibodies.

Photo-Acoustic

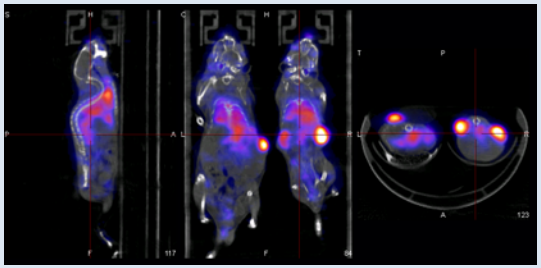
(Vevo LAZR; VisualSonics,



Imaging Oxygen saturation (SO_2).

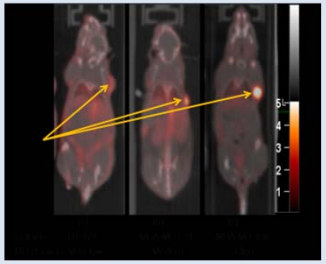
NUCLEAR

NanoSPECT/CT
(Bioscan, Inc.)



[^{111}In]Panitumumab
Fused SPECT (Color)/CT (grey)
Animal Model: athymic nudes MDA-MB-231 cells)

Inveon μPET/CT
(Siemens Medical)

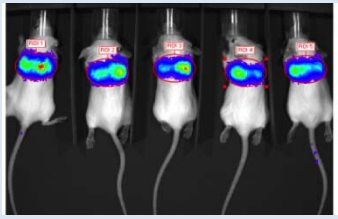


[^{89}Zr]-Panitumumab HER1 probe (PET/CT)

OPTICAL

Bioluminescence

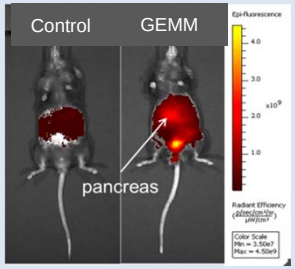
Xenogen IVIS SPECTRUM
(Caliper Life Sciences)



Cell Trafficking

Fluorescence

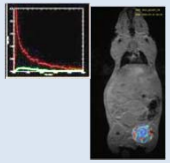
Maestro-GNIR (Cambridge Research Institute); FMT 2500 (Tomographic Fluorescence) (PerkinElmer/VisEn Medical)



A protease activatable fluorescent *in vivo* imaging agent (ProSense 750) in a murine GEM model for pancreatic adenocarcinoma.

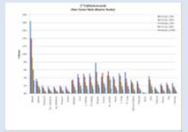
Archive

Image Analysis



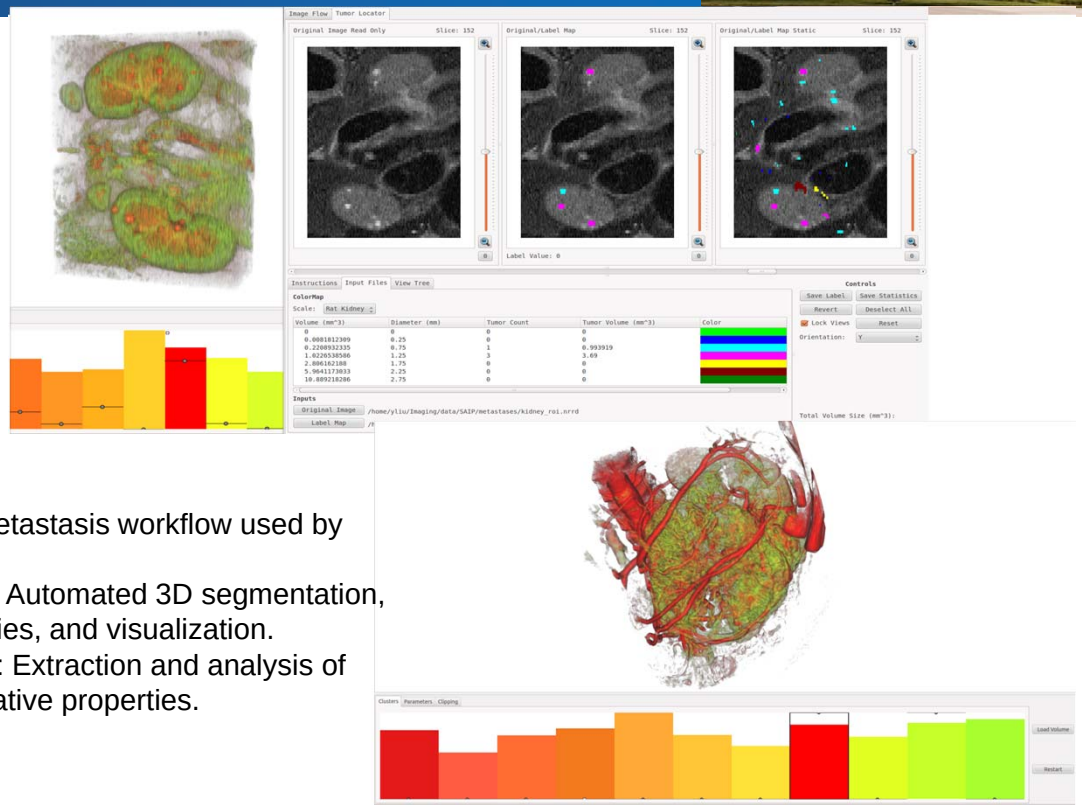
High-end workstations
Remote access to analysis programs

Autoradiography & Biodistribution

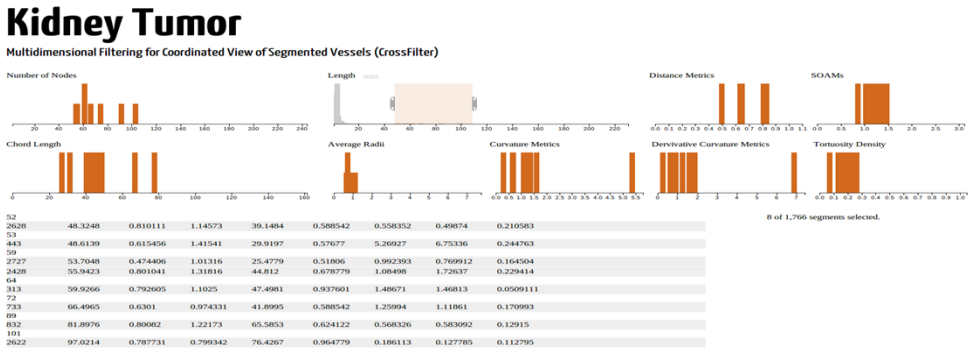


Imaging and Visualization Group (IVG): Focused on algorithms, software, and workflows for rapid and reproducible analysis of biological images.

- Automated algorithms and workflows to facilitate reproducible analyses and increase throughput for quantitative analyses of tumor volumes, metastases, and measured properties.
- Designed for ease of use by SMEs rather than computer scientists
- Algorithms and workflows for both 2D sections and 3D volumes.



Top: Metastasis workflow used by SAIP.
Middle: Automated 3D segmentation, properties, and visualization.
Bottom: Extraction and analysis of quantitative properties.



FNL/ISP/ABCC Jack Collins, Yanling Liu