Design, Processing, Analysis, and Reporting of Longitudinal Small-Animal SPECT Studies

inviCRO

November 6, 2012

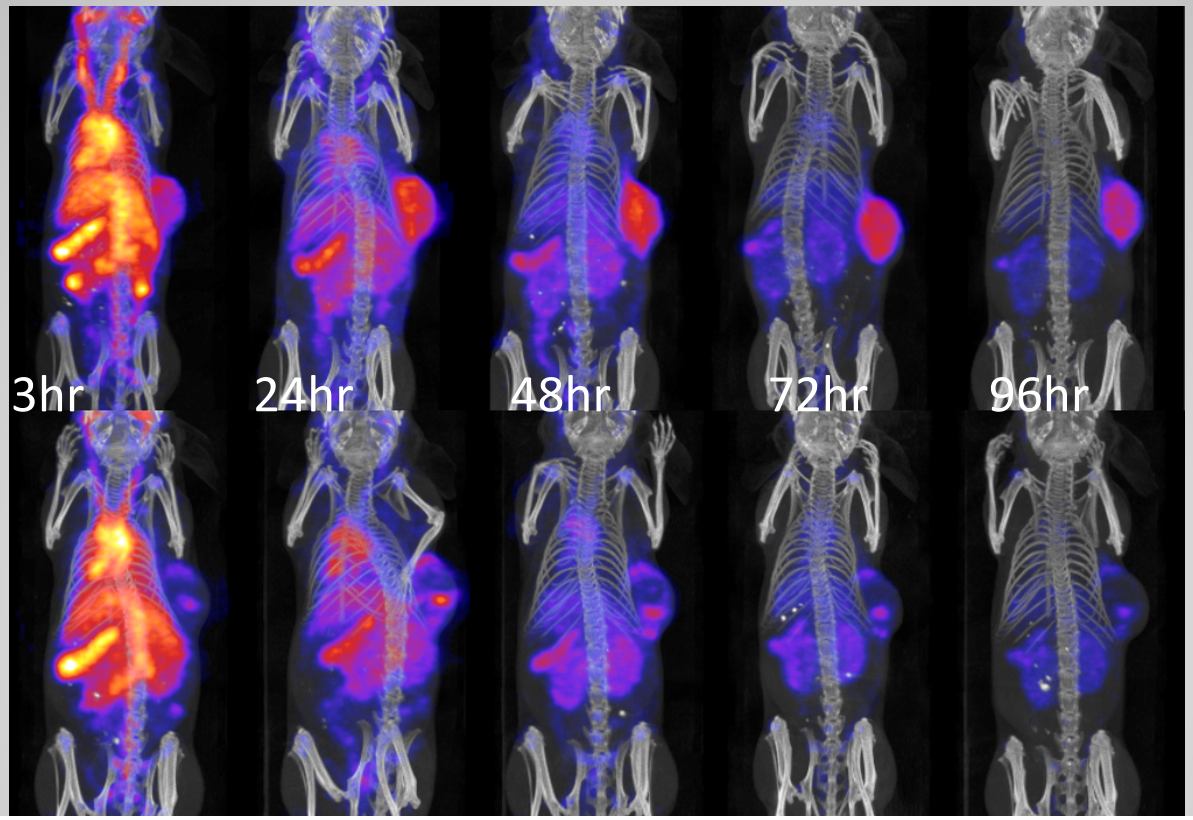
Introduction

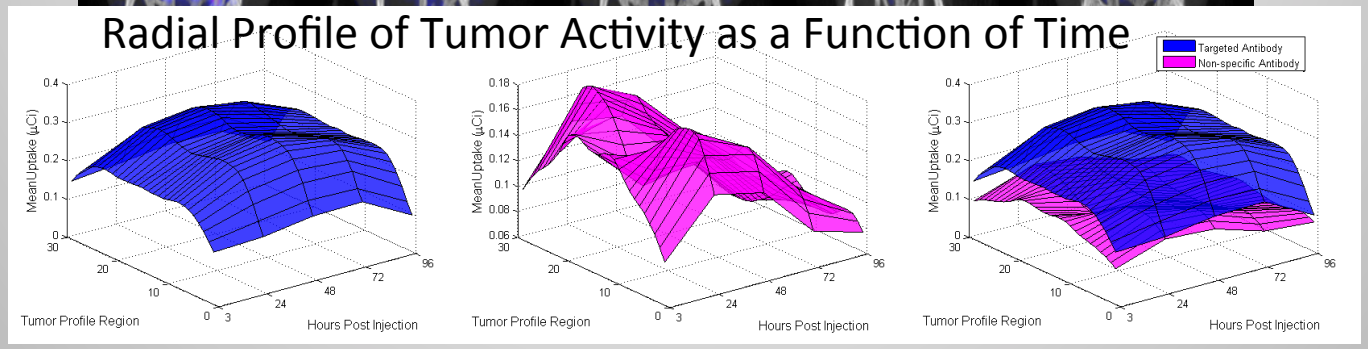
- Founded in 2008, headquartered in downtown Boston with a satellite office in Cologne, Germany
- Research team comprised of a broad range of technical disciplines including advanced degrees in: physics, optics, software engineering, electrical engineering, chemical engineering, mathematics, neuroscience, biomedical engineering and statistics.
- Three primary departments, united to execute successful contract imaging studies, advanced image analysis, and image data management
- Services
  - Full-scale image study management – 12+ studies per month
  - MSAs with 7 validated partner facilites
  - Image analysis services – 35,000+ scans per year
  - Broad application experience: oncology, neuroscience, toxicology, immunology, ...

Introduction

- iPACS® and VivoQuant™ Software
  - VivoQuant™ analysis and viewing software – installed at 100+ imaging labs world-wide
  - iPACS® data management and archiving solution – installed at 8 top pharmaceutical companies
  - Developed to work with all preclinical imaging platforms and clinical DICOM data
- Growth
  - Supporting four clinical trials with imaging endpoints
  - Advanced the iPACS® platform for GLP storage of pharmaceutical industry medical and histological imaging data
  - 2013 opening of a new laboratory with multi-modal imaging, radiochemistry, and autoradiography

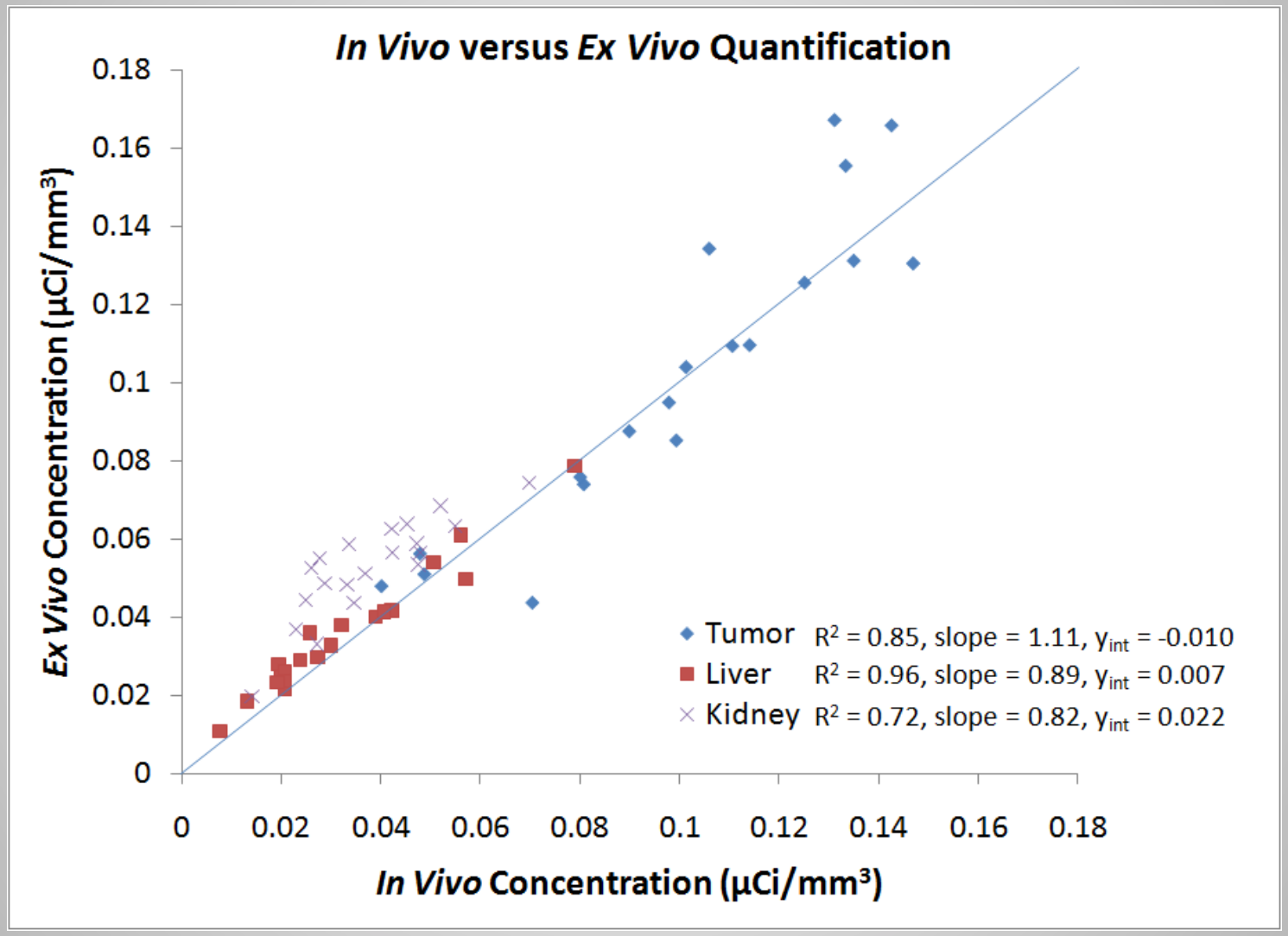
Introduction





Example results from an 111In antibody tumor study.
Cover of JPET, May 2011, Review on imaging antibodies in mice

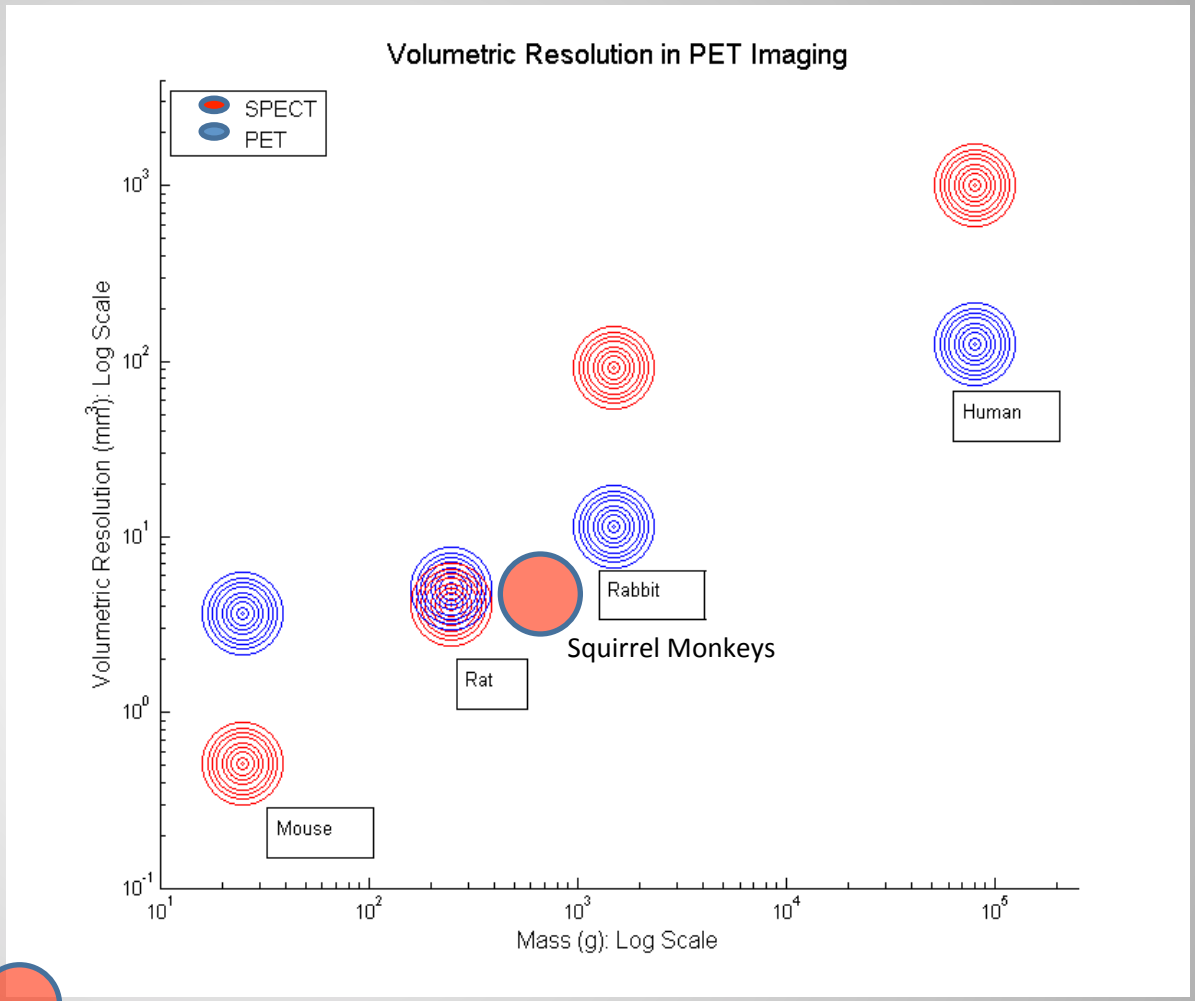
Introduction



Introduction

Understanding the range of sensitivities and resolutions available with *in vivo* and *ex vivo* scanning modalities

Dependencies:
- Isotope Selection
- Radiochemistry
- Pharmacokinetics
- Radiation Dosimetry
- System Manufacturer
- Acquisition Protocol



Course Outline

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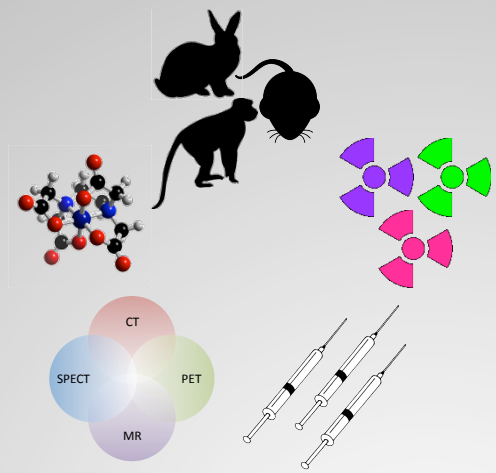
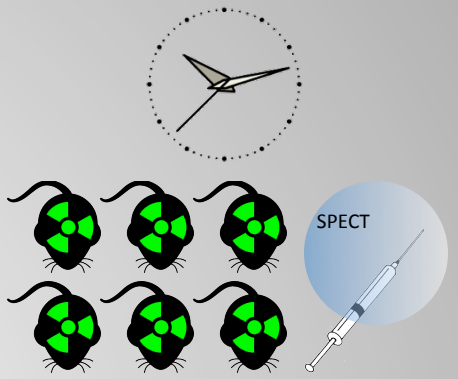
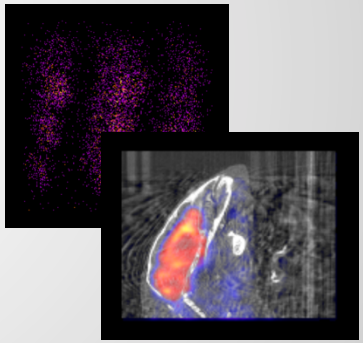
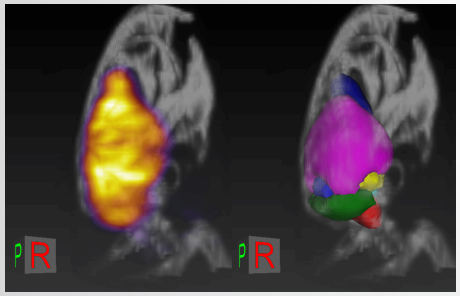


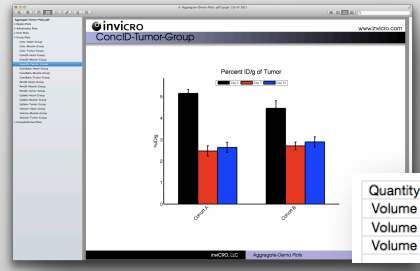






iPACS

	Inj Dose (uCi)	Tumor Volume (mm^3)
0	206.065	130.792
0	210.739	201.368
0	209.5	196.288
Cohort A	0	180.346	151.648
Cohort A	0	195.196	180.984
Cohort A	0	184.387	162.192
Cohort A	0	211.943	104.664



Quantity	Organ	Group	Sample 1	Sample 2	Paired t-test	P-Value	Result	Comment
Volume	Tumor	All Subjects	Day 0	Day 7	Two-tailed	6.77E-08	Mean B > Mean A	VHS
Volume	Tumor	All Subjects	Day 0	Day 14	Two-tailed	1.57E-06	Mean B > Mean A	VHS
Volume	Tumor	All Subjects	Day 7	Day 14	Two-tailed	0.0115557	Mean B > Mean A	S

Using a longitudinal SPECT/CT study to go from biological question to $p < 0.01$.

Study Design

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What is the biological question?

- → What are the pharmacokinetics of a liposome/protein/peptide?
- → How do the pharmacodynamics of a novel therapeutic affect the pharmacokinetics of a well-characterized radiopharmaceutical?
- → What is the tumor-to-liver ratio of a compound as a function of time?
- → What is the relationship between myocardial infarct volume and ejection fraction?
- → Is the binding affinity for a compound different between aged and young animals?
- → How do changes in mass dose impact blood clearance?

The answer is **not**: “Let’s put in FDG and see where it goes.”

Study Design

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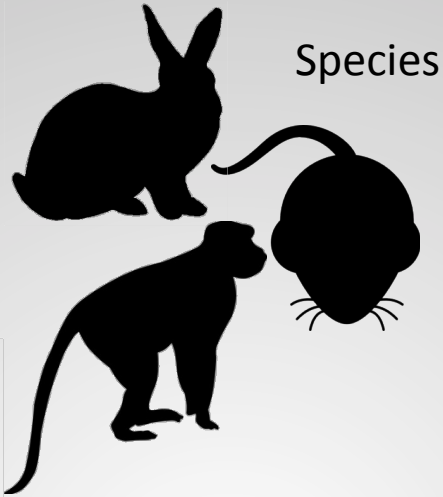
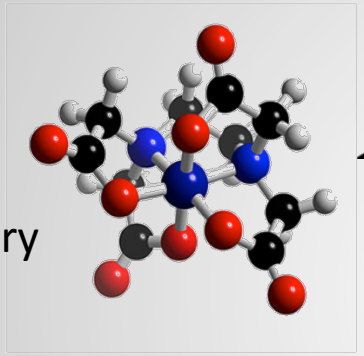
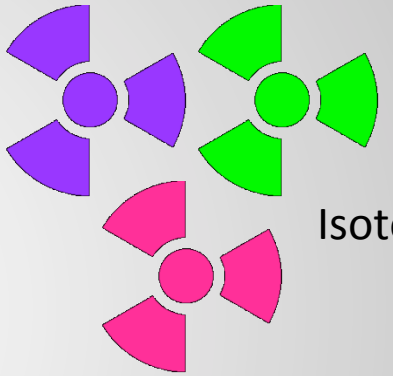


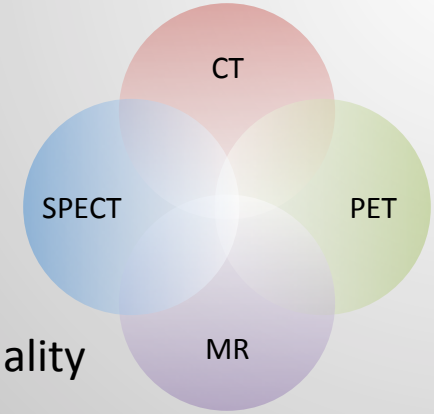
Species and Model



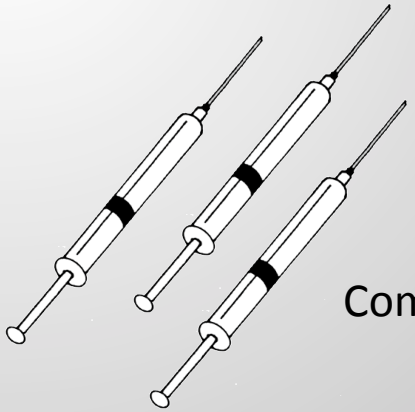
Radiochemistry



Isotope Selection



Imaging Modality



Compound

The evaluation criteria for each of these design elements and their combination is signal-to-noise or signal-to-background ratio.

Study Design

Example: How isotope selection affects signal.

Signal should be significantly* greater than background for visualization.

For an example system (NanoSPECT/CT), typical background values are in the range of 0.1-0.5 uCi/mL (depending on isotope, scan duration, and radioactivity distribution).

Therefore, assuming one isotope per molecule, the concentration of radiolabeled compound must be > ~100 pM for I125 but only > 5-10 pM for I123.

Example Calculation:

Background = ~0.2 uCi/mL

Specific activity (assuming one I125 per molecule) = 2199 uCi/nmol

Let Activity Concentration = Background = 0.2 uCi/mL

Activity Concentration / Specific Activity = [0.2 uCi/mL] /[2199 uCi/nmol] = 90 pM

Thus, ~90 pM concentration of I125-labeled compound is indistinguishable from background.

*Conventional wisdom states that a factor of 2.5 to 3 is deemed appropriate for signal detection tasks by human observers; there are many caveats given the context of the task.

Study Design

Example: How isotope selection affects signal.

Isotope	Specific Activity (uCi/nmol)	Background (uCi/mL)	Compound Conc = Background (nM)	Compound Conc = Background (nmol/mL)
125I	2200	0.2	0.1	0.0001
123I	235000	0.2	0.001	0.000001
99mTc	520000	0.2	0.0004	0.0000004
111In	47000	0.3	0.006	0.000006

Approximate theoretical concentration required to achieve SNR = 1.
Assumes one isotope per molecule.

SPECT Isotopes	^{67}Ga	^{123}I	^{125}I	^{111}In	^{177}Lu	^{99m}Tc
Half-Life (days)	3.26	0.55	59.42	2.80	6.75	0.25
Energy in keV (mean no. of gamma emissions per 100 decays)	93.2 (42.4), 184.6 (21.2), 300 (16.6)	28.0 (86.7), 159.0 (83.0)	27-32 (144.6)	171.3 (90), 245.4 (94.0)	56.7 (5.4), 112.9 (6.4), 208.4 (11.0)	140.5 (89)
Recon. Resolution (mm^3) 1mm pinh. w/Jasz	0.73	0.51	1.00	0.42	0.51	0.41
Avg. Sensitivity in 20g mouse (cps/ uCi/cc, 1.0mm pinh array)	0.182	0.442	0.443	0.285	0.052	0.226
Avg. Sensitivity in 20g mouse (cps/ uCi/cc, 2.0mm pinh array, ~microPET resolution)	0.73	1.77	1.77	1.14	0.21	0.90
Whole-body Absorbed Dose (Gy) per 100 µCi and a 72 hr biological half life (max dose - no biological clearance)	0.258 (0.542)	0.065 (0.077)	0.276 (5.726)	0.284 (0.556)	1.117 (3.646)	0.017 (0.019)
Specific Activity (theoretical) Ci/ µmol	40	237	2.2	47	19	522
Radioisotope/Antibody[a]	0.00375	0.005-0.008	0.01-0.3	0.02-0.16	0.01-0.1 imaging, max 2.4 therapy	0.003-0.008
Primary Labeling Method	DOTA[f]	direct labeling (iodination)	direct labeling (iodination)	DTPA[f]	DOTA[f]	HYNIC[f], MAG3[f], hexahist-adine
Primary Strength/Use	Translatable to Ga-68 for PET	Neuro-imaging	Long half-life	Availability and Translation	Imageable and Therapeutic	Low-Priced
Primary Shortcoming	Contaminat-ion with Zn	Cost, Half-life	Long half-life	High cost	Potential Therapeutic Effect	Short half-life
Cost/mCi ($)[b]	+++	+++	++	+++++	++	+

Study Design

Example: How tissue uptake kinetics affect signal.

Tissue uptake is a function of:

- Blood flow rate = $Q(1-H)/f_{free}$
- Extravasation rate = $2PR_{cap}/R^2_{Krogh} = PS/V$
- Interstitial diffusion rate = $D\varepsilon/R^2_{Krogh}$
- Binding or metabolism rate = k_{rxn}

where f_{free} = fraction of drug unbound in plasma, H = hematocrit, Q = blood flow rate (volume of blood per volume of tissue per time), P = capillary permeability, S = capillary surface area, V = tissue volume, ε = void fraction, D = diffusion coefficient, R_{Krogh} = capillary-to-capillary half-distance

Each of these four transport/kinetic rates may be estimated from target and compound properties to determine the rates that most/least affect uptake.

The rate-limiting step(s) will determine the meaning of “signal”.

Study Design

Example: How tissue uptake kinetics affect signal.

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- Binding or metabolism rate = k_{rxn}

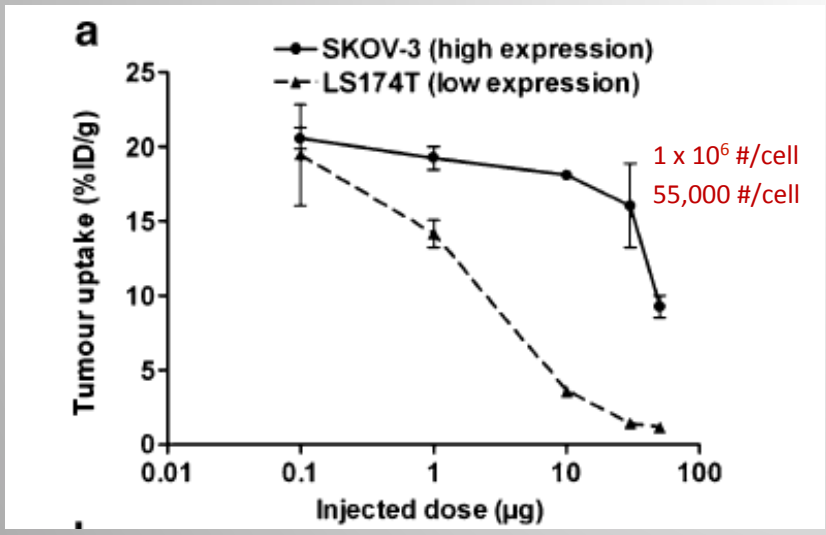
Estimations from literature values	Oxygen	18FDG	Antibody (sub-saturating)	Antibody (saturating)
Blood flow (s^{-1})	0.09	0.06	0.06	0.06
Extravasation (s^{-1})	1.4	0.003	0.00001	0.00001
Diffusion (s^{-1})	0.3	0.04	0.0004	0.0004
Binding/metabolism (s^{-1})	0.2	0.0006	0.07	0.000001

Slowest rate determines the meaning of “signal”. Oxygen is a measure of blood flow, 18FDG is a measure of glucose metabolism, sub-saturating antibody a measure of extravasation/ vascularity and saturating antibody a measure of antigen binding.

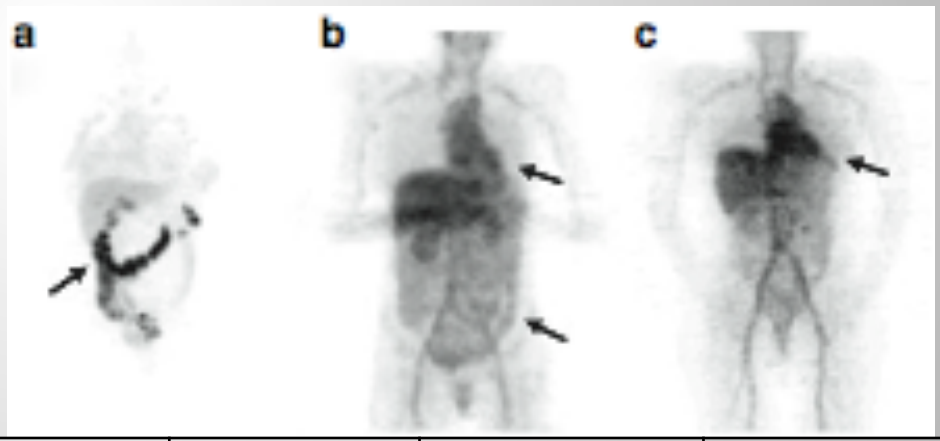
Study Design

Specific Activity and Mass Dose.

- Maximum specific activity (= lowest injected mass dose) depends on:
  - Isotope
  - Isotope/ligand (labeling chemistry)
  - Can always add cold compound to lower specific activity
- Competition also factors into selection of specific activity and dose
- Specific activity determines lowest antigen expression level detectable
- Typically, lower injected mass dose is preferable, particularly for peptides and other small molecules, but not always the case.



111In-labeled anti-HER2 affibody
Tolmachev et al. 2011



	Cohort 1 (a)	Cohort 2 (b)	Cohort 3 (c)
^{89}Zr-Herceptin	1.5mg	1.5mg	1.5mg
Herceptin	8.5mg	48.5mg	6mg therapy + 8.5mg

Increasing Herceptin from 8.5mg; 48.5mg slowed blood clearance & improved tumor accumulation of ^{89}Zr-Herceptin as did 8.5mg when the subject was on Herceptin (Dijkers, et. al. 2010)

Study Design

Number of animals.

How can the study's longitudinal-ism be incorporated into statistics?

Scheibe, Nuc. Med. Bio. 35 (2008) 3-9

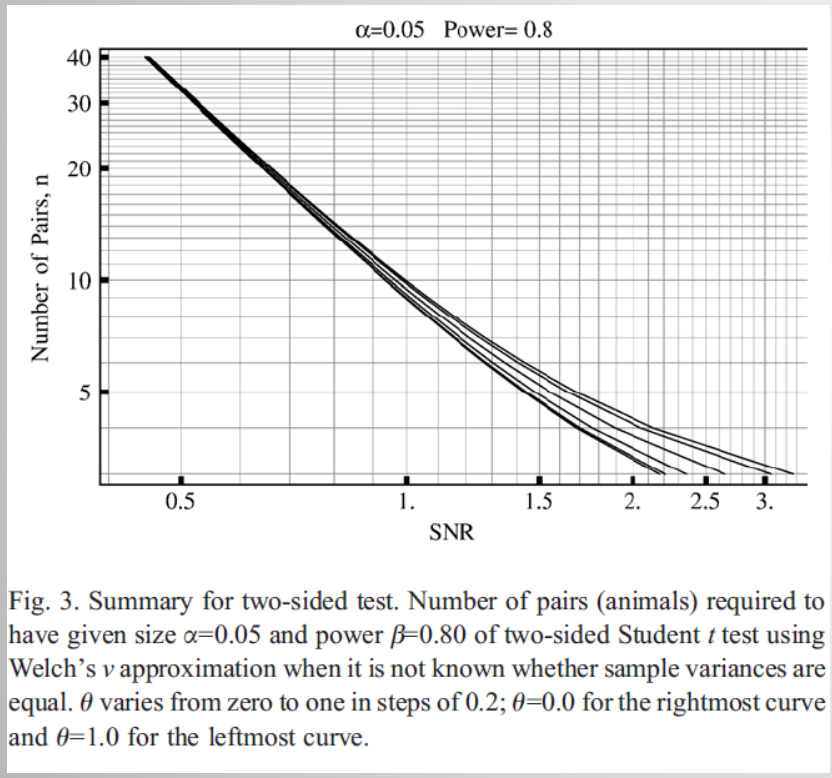
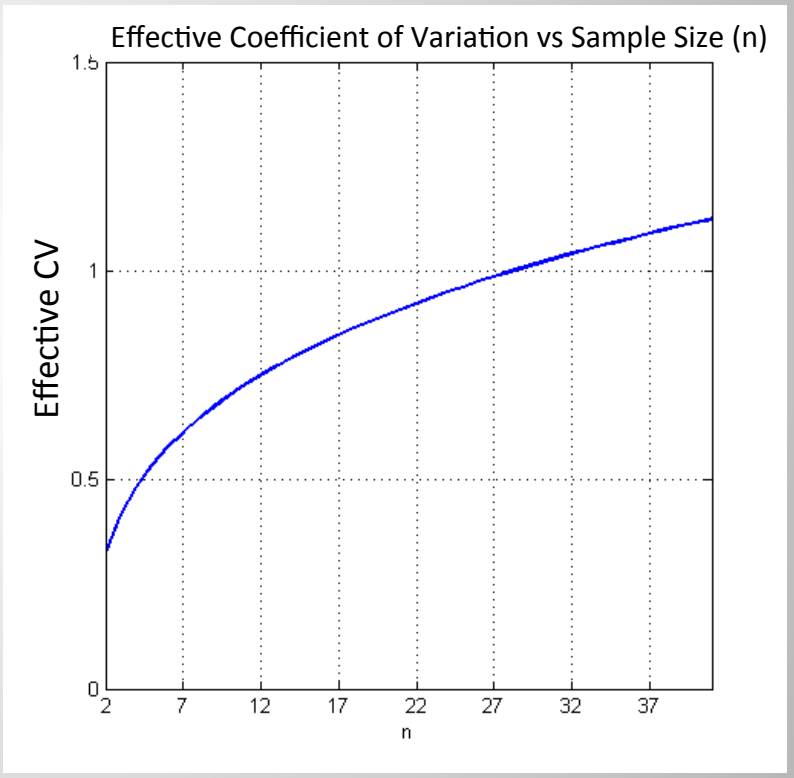


Fig. 3. Summary for two-sided test. Number of pairs (animals) required to have given size α=0.05 and power β=0.80 of two-sided Student t test using Welch's v approximation when it is not known whether sample variances are equal. θ varies from zero to one in steps of 0.2; θ=0.0 for the rightmost curve and θ=1.0 for the leftmost curve.



$$\mathrm{SNR} = \frac{|\mu_1 - \mu_0|}{(\sigma_1^2 + \sigma_0^2)^{\frac{1}{2}}}$$

$$\mathrm{CV}_{eff} = \frac{(\sigma_1^2 + \sigma_0^2)^{\frac{1}{2}}}{|\mu_1 - \mu_0|}$$

Study Design

Number of animals.

How can the study's longitudinal-ism be incorporated into statistics?

Building on the work of Eckelman et. al., and Scheibe, we may use a one-way MANOVA to estimate the required effect size necessary to expect significance at a fixed significance level (alpha) and power (1-beta). Conversely, given an anticipated effect size, the number of animals required may be estimated.

$$\Delta R = \sqrt{\frac{2\delta_p}{n(\mathbf{1}^t\mathbf{D}^t\mathbf{P}^{-1}\mathbf{D}\mathbf{1})}} \quad (1)$$

where ΔR is the change in the effect and n is the number of animals.

D is a scaling transformation matrix whose elements are the reciprocals of the standard deviations for each feature and **P** is a p x p population correlation matrix on the features (solved from $\mathbf{P} = \mathbf{D}\boldsymbol{\Sigma}\mathbf{D}^t$ where **Σ** is the class covariance matrix).

δ_p is a non-centrality parameter given by $\delta_p = \frac{n}{2}(\boldsymbol{\mu}_1 - \boldsymbol{\mu}_2)^t\boldsymbol{\Sigma}^{-1}(\boldsymbol{\mu}_1 - \boldsymbol{\mu}_2)$ that also depends on the selection of alpha and beta.

Study Design

Number of animals.

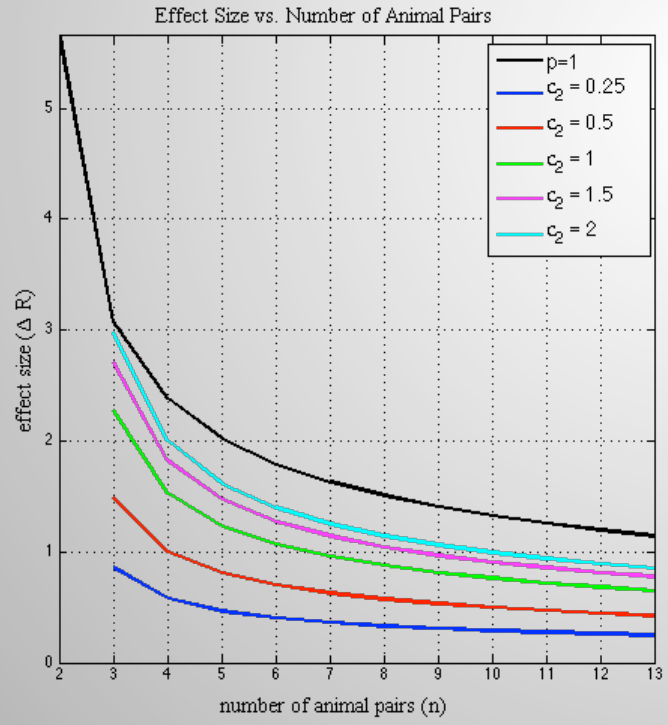
Special Case: One Feature:

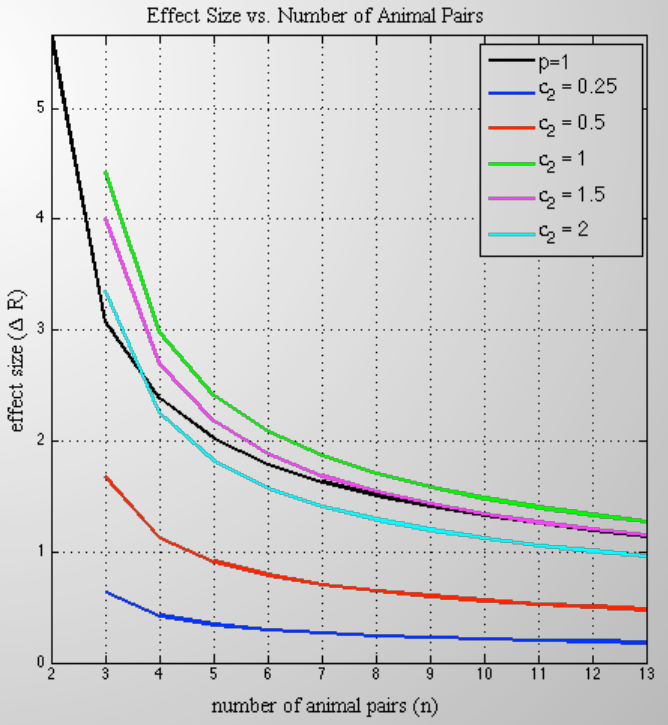
$$\Delta R = \sqrt{\frac{2\sigma_1^2\delta_1}{n}} \quad (2)$$

Special Case: Two Features:

$$\Delta R = \sqrt{\frac{2\sigma_1^2}{n}\left(\frac{\gamma^2(1-\rho^2)\delta_2}{\gamma^2 - 2\rho\gamma + 1}\right)} \quad (3)$$

ρ = -0.5





ρ = 0.9

Study Design

Mathematical modeling

Mathematical modeling has widespread use in imaging and other drug development areas, focusing primarily on assessing either the pharmacokinetics (PK) or pharmacodynamics (PD) of a drug within the body.

- The pharmacokinetic phase encompasses all the events between the administration of a dose and the achievement of drug concentrations throughout the body.
- The pharmacodynamic phase encompasses all the events between the arrival of the drug at its site of action and the onset, magnitude, and duration of the biological response.

In imaging, compartmental models are typically applied to describe pharmacokinetic features like:

- Blood clearance (drug metabolism and excretion)
- Irreversible trapping in tissues (i.e., Patlak analysis)
- Reversible trapping in tissues (i.e., Logan analysis)

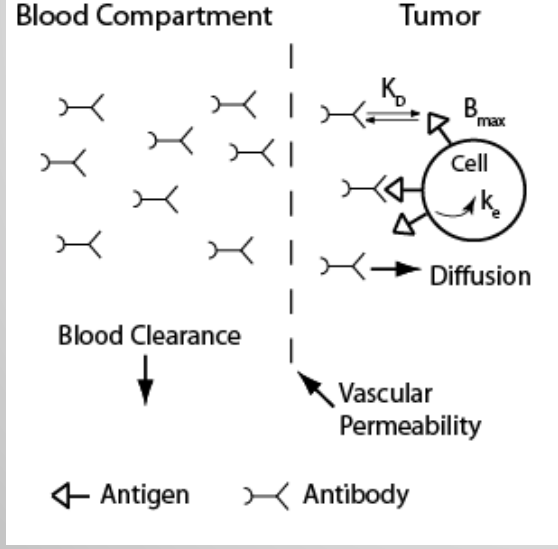
Basic Pharmacokinetics and Pharmacodynamics, Rosenbaum, Wiley 2011

Study Design

Mathematical modeling

Models, particularly compartmental models, are often used to assess image data post-acquisition (i.e., estimation of kinetic parameters). Models, especially mechanistic models, may also be used in a predictive sense.

For example, models such as this tumor model based on the Krogh cylinder model may be used to help guide preclinical study design, including parameters such as dose range and specific activity, given some criteria based on clinical need and antigen/target properties.



Baxter and Jain, Br J Cancer, 1996
Jackson et al. Br J Cancer, 1999
Thurber et al. JNM, 2007

- Krogh distributed model
  - oxygen transport in tissue (1919)
- Model assumptions
  - No convection - high tumor pressure
  - Vascular tumor

Study Design

Mathematical modeling

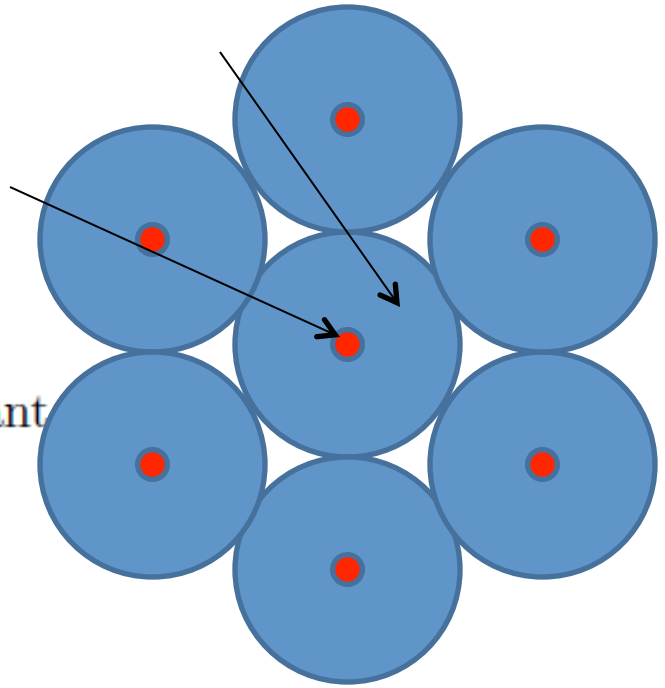
$$\frac{\partial Ab(\vec{x},t)}{\partial t} = D \cdot \nabla^2_{\vec{x},\vec{x}}(Ab(\vec{x},t)) - \frac{k_{on}}{\epsilon} \cdot Ab(\vec{x},t) \cdot Ag(\vec{x},t) - k_{off} \cdot B(\vec{x},t)$$

$$\frac{\partial B(\vec{x},t)}{\partial t} = \frac{k_{on}}{\epsilon} \cdot Ab(\vec{x},t) \cdot Ag(\vec{x},t) - k_{off} \cdot B(\vec{x},t) - k_e \cdot B(\vec{x},t)$$

$$\frac{\partial Ag(\vec{x},t)}{\partial t} = R_s - \frac{k_{on}}{\epsilon} \cdot Ab(\vec{x},t) \cdot Ag(\vec{x},t) + k_{off} \cdot B(\vec{x},t) - k_e \cdot Ag(\vec{x},t)$$

$$\frac{\partial I(\vec{x},t)}{\partial t} = k_e \cdot B(\vec{x},t) - k_{resid} \cdot I(\vec{x},t)$$

$Ab(\vec{x},t)$ = free antibody
$B(\vec{x},t)$ = bound antibody
$Ag(\vec{x},t)$ = antigen concentration
$I(\vec{x},t)$ = internalized
k_{on} = antibody binding rate constant
k_{off} = antibody dissociation rate constant
ϵ = effective void fraction
k_e = endocytosis rate constant
R_s = antigen synthesis rate
k_{resid} = residualization rate constant



Study Design

Mathematical modeling

Image Study Parameters			
Animal weight	Body weight	25 g (mouse)	
Activity	Injected activity	100-1000 uCi	Measure syringe activity before and after injection
Dose	Mass of compound injected	0.001-250 ug	Determine mass of injected compound (i.e. by absorbance)
Contrast Agent	Radiolabel/fluorophore	^{111}In, ^{125}I, ^{99m}Tc, etc.	
Tumor size	Mass of tumor	0.1-1 g	CT
A	Fraction of alpha phase blood clearance	0.5-0.9	Blood activity over time or ELISA
$t_{1/2,alpha}$	Half-life of alpha phase blood clearance	1-12 h	Blood activity over time or ELISA
$t_{1/2,beta}$	Half-life of beta phase blood clearance	3-48 h	Blood activity over time or ELISA

Study Design

Mathematical modeling

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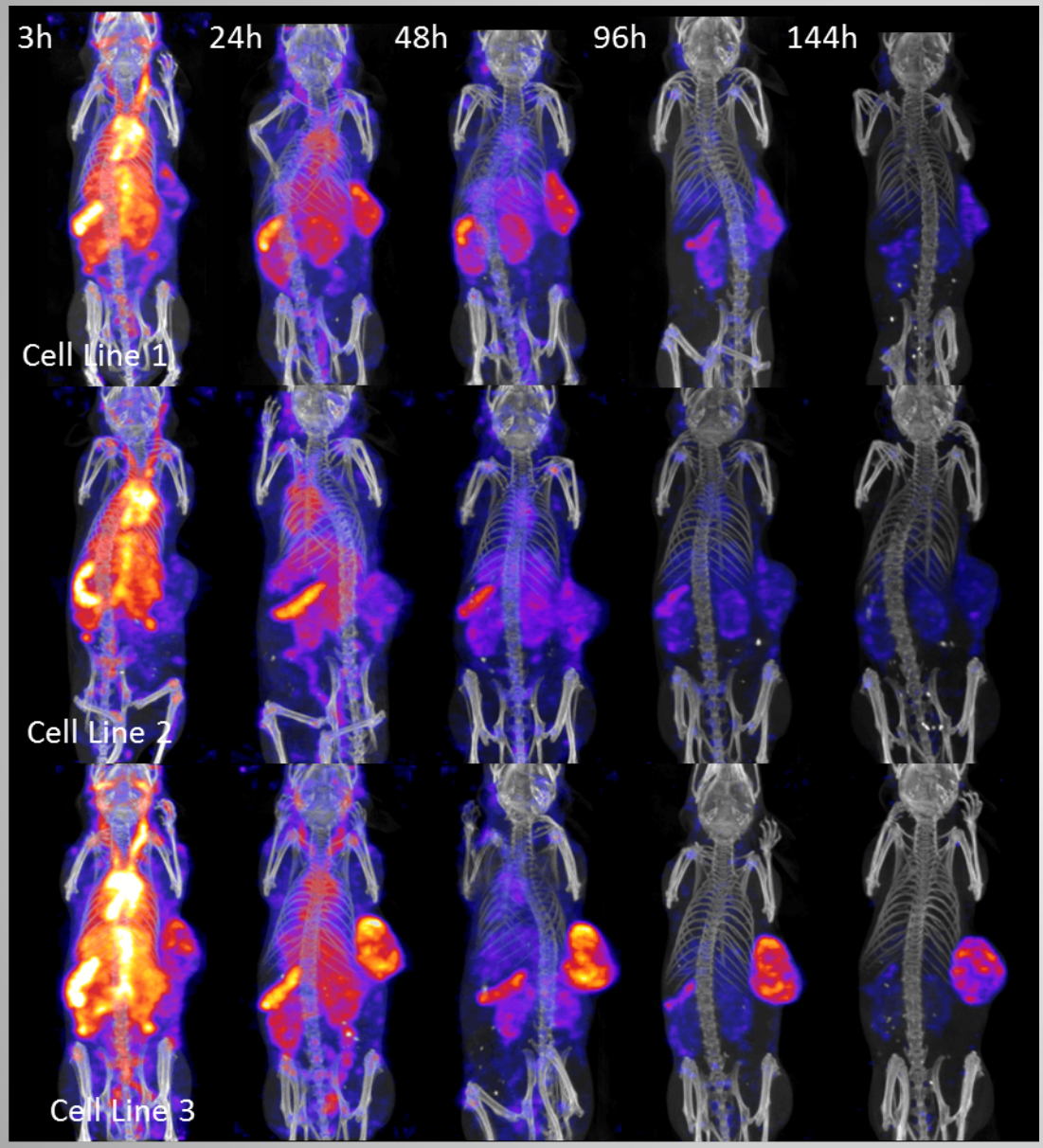
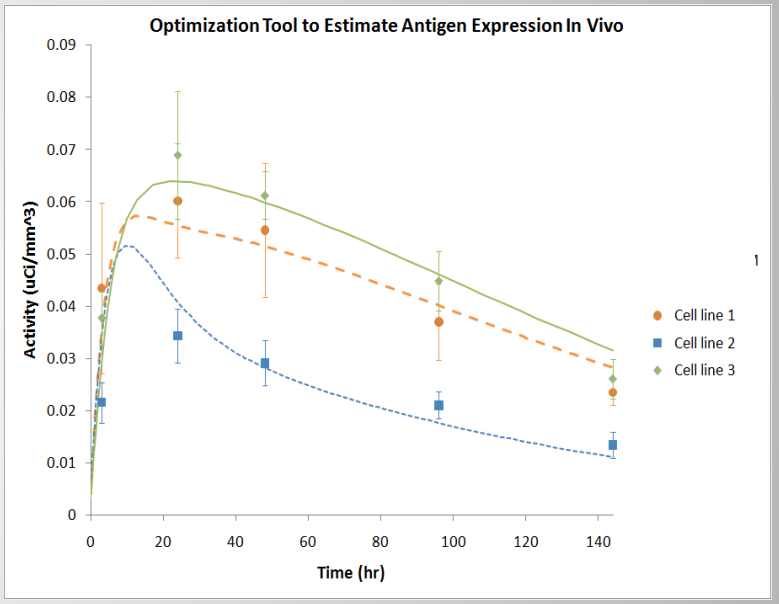
Parameter Inputs to Model

Parameter	Description	Typical Range	Assay
Ligand/Target Parameters			
K_D	Affinity	0.1 – 10 nM	Competitive cell binding (with radiolabeled or fluorescent compound)
MW	Molecular Weight	150 kDa (mAb)	
$t_{1/2,ke}$	Half-life of cellular internalization	3-15 hours	Cell uptake over time (with radiolabled or fluorescent compound)
B_{max}	Antigen surface density	10^3-10^6 #/cell	Competitive cell binding (with radiolabled compound or fluorescent compound with known standard)
ρ_{cell}	Cell density	10^8-10^9 cells/mL	Ex vivo counting of Hoechst-stained nuclei in a known volume of tissue
R	Half-distance from capillary to capillary (measure of vascularity)	20 – 150 µm	Ex vivo immunohistochemistry with CD31 staining
$t_{1/2,kresid}$	Residualization rate	12-120 hours	Release of internalized compound over time (with radiolabeled compound)

Study Design

Mathematical modeling – oncology antibody example.





Estimation of B_{max} and vascularity in three different tumor lines.

Study Design

Other Considerations

Effect of molecular size – small and large are better than "medium".

Radiolabeling and ligand binding/function

- Assay of cold-labeled materials

Clinical Translation

- How does the isotope/radioactive dose selection affect translatability

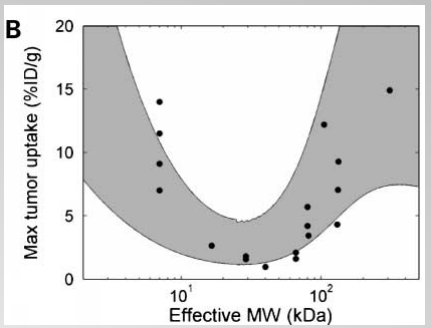
Specific activity and mass dose

- Does ligand bind? Is drug accumulation saturable?
- Does ligand exhibit off-target binding?
- How do metabolism/clearance/tissue distribution change with dose?
- Dose escalation studies are critical

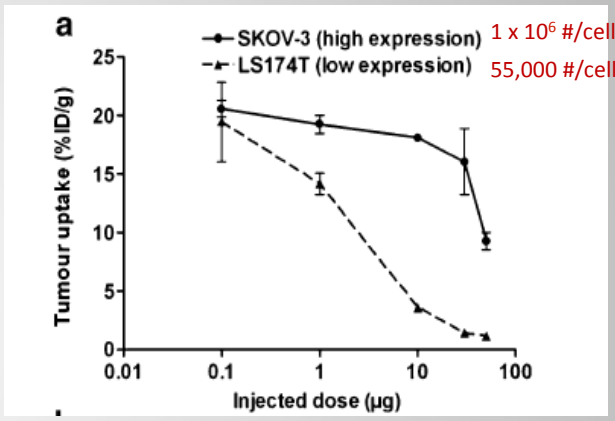
Scaffold selection

- bsAb, IgG, minibody, diabody, scFv, affibody, peptide

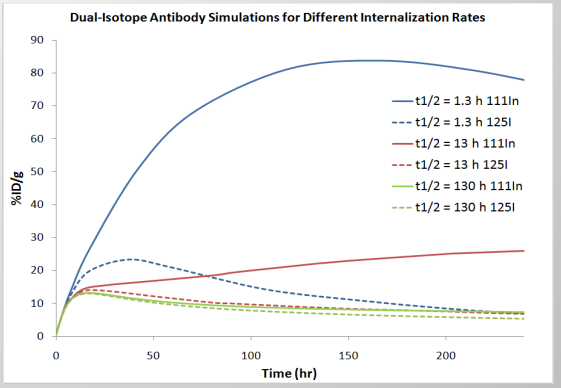
Internalization Rates



HER2 binding agents, Schmidt and Wittrup, 2009.



111In-labeled anti-HER2 affibody, Tolmachev et al. 2011



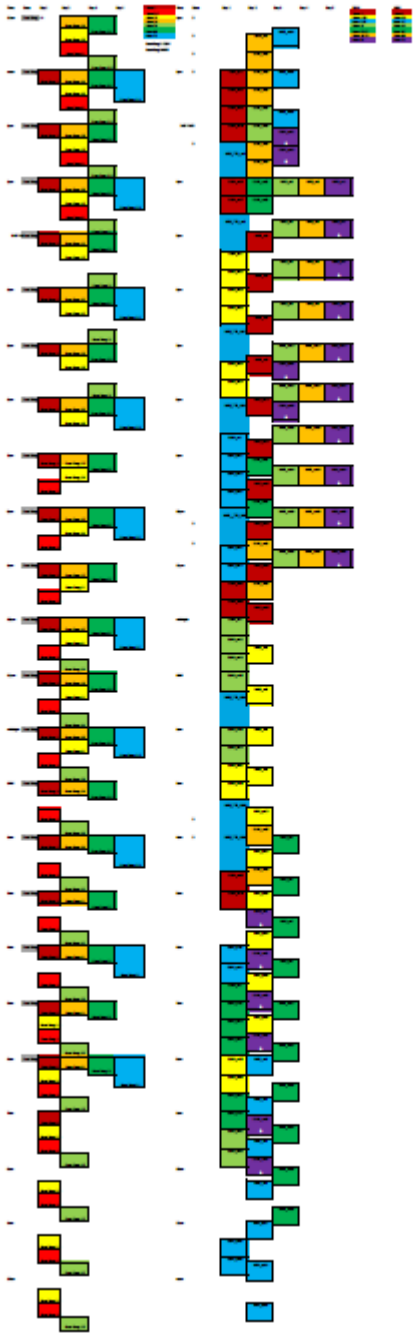
Model simulation of %ID/g over time for antibodies with different internalization rates (In111, I125)

Study Management

Managing time.

- 274 SPECT/CTs over 7.5 days of around-clock-imaging
- Two injections of ~700-800uCi of I125-labeled antibody at 0 and again 74 or 97 hrs.
- Ab1: 1, 8.5, 16, 24, 36, 48, 75, 78.33, 81.67, 85, 91.67, 98.33, 110, 134
- Ab2: 1, 8.5, 16, 24, 36, 48, 72, 98, 105.5, 113, 121, 133, 145, 169
- 16 scientists/technical people

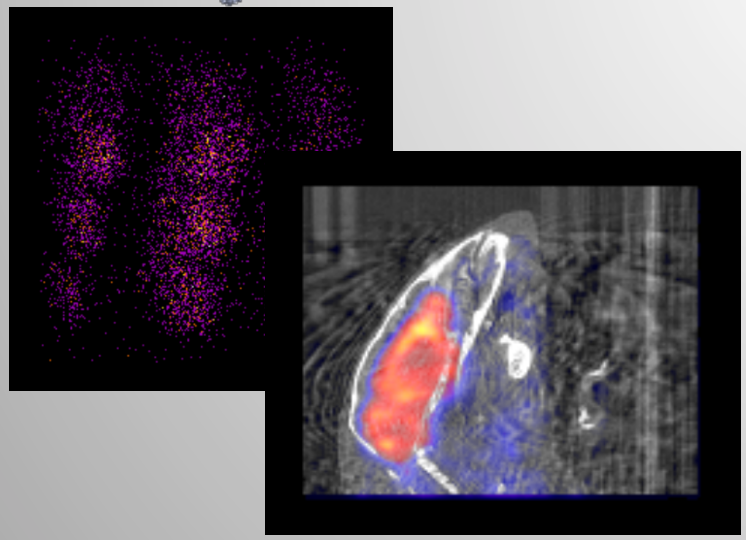
- Tumor growth
- Radioactive decay
- Scanner time
- Personnel hours
- Compound stability



Study Management

Managing time and distance.

- Tumor growth
- Radioactive decay
- Scanner time
- Personnel hours
- Compound stability



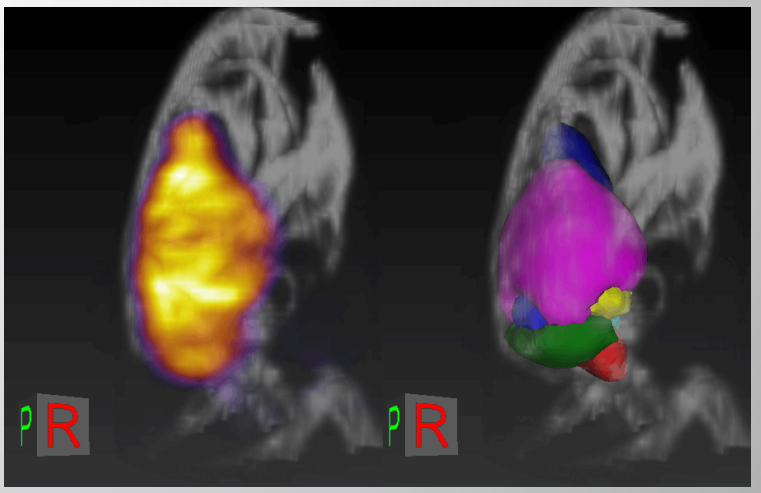


Image Analysis

Data Formats

invicro
imaging services and software

Example Clinical Systems

- GE Symbia
- GE Discovery
- GE Biograph
- Mediso AnyScan
- Philips Ingenia
- Philips BrightView
- Philips GEMINI
- Siemens Discovery
- Siemens Definition
- Siemens Magnetum
- …

Example Preclinical Systems

- ASPECT MR
- Bioscan NanoSPECT
- Bruker MR
- Caliper IVIS Optical
- GE Locus
- Mediso NanoScan
- Milabs uSPECT
- ScanCo CT
- Siemens Inveon
- SkyScan CT
- Varian MR
- …

Clinical Data

DICOM

Preclinical Data

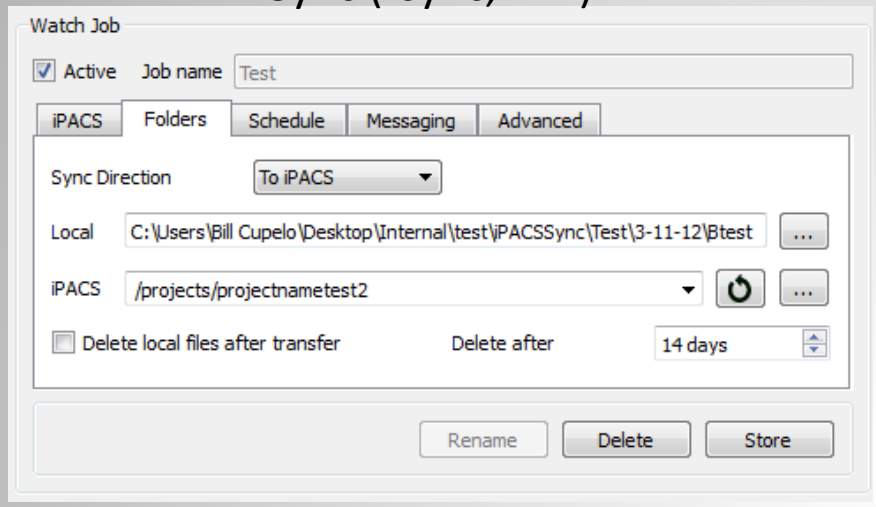
- AIM
- AVW
- BRUKER
- DICOM
- IMG/HDR
- MHD/MHA
- NIFTI
- RAW
- TIFF

DICOM data are standard, but many other formats require support.

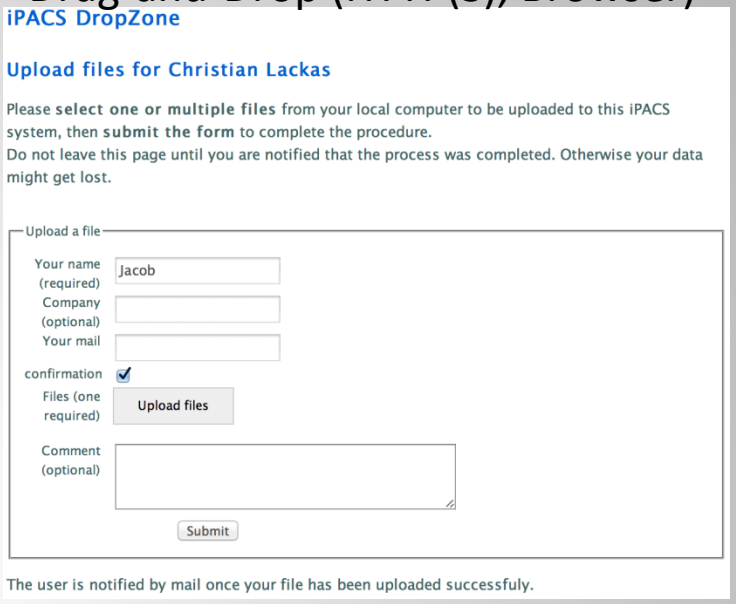
Image Analysis

Gathering the data

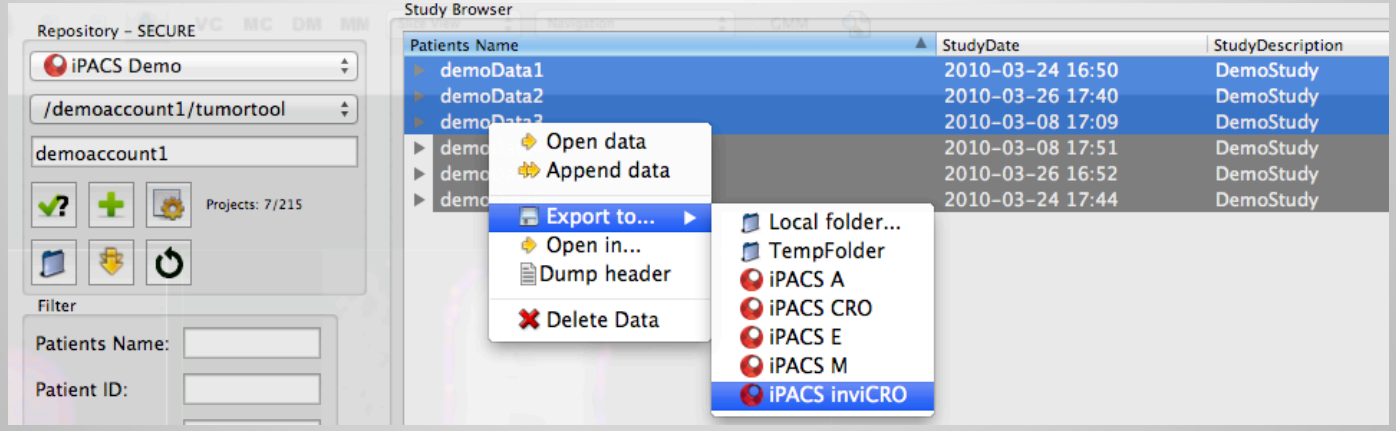
Sync (rsync, FTP)



Drag-and-Drop (HTTP(S), Browser)



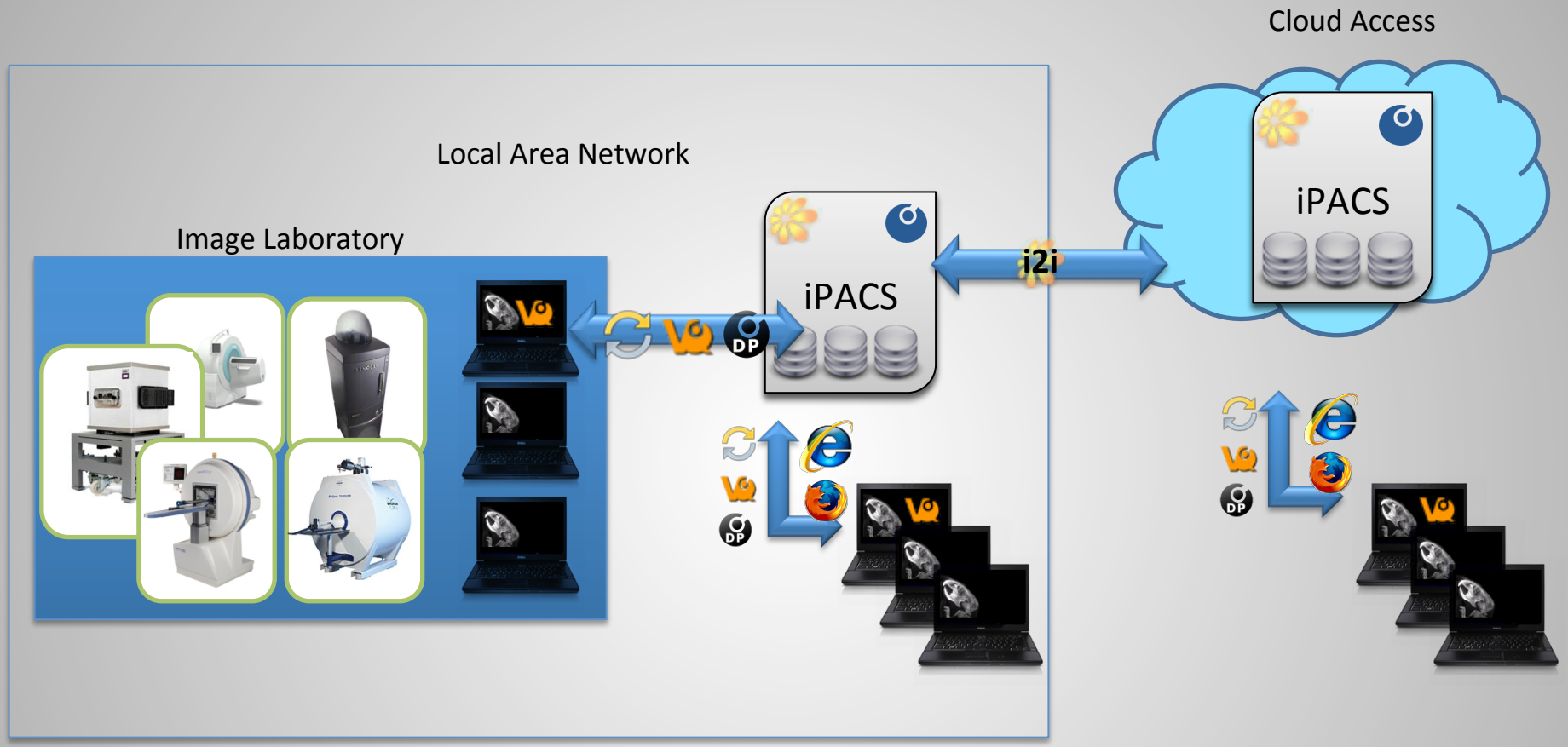
Direct DICOM transfer



Data transfer/upload options: DropBox, syncplicity, Cubby, huddle, ShareFile, iPACS

Image Analysis

Gathering the data



iPACS Sync: Keep local and remote folders in sync, secure transfer, optimized for large data sets

VivoQuant/InVivoScope provides full access to all iPACS data, integrated workflows

DICOM Proxy: Connect any DICOM client to the iPACS, e.g. DICOM export of imaging equipment, DICOM viewer, etc…

Image Analysis
Organization

192.168.1.139/browser?project=30

Project » iPACS » TherapeuticTrack1 » ProjectD »PET_CT (/therapeutictrack1/projectd/pet_ct) [open]

[Reload] [WebDisk] mode: [non recursive]

Select All | Clear

Sel	Patients Name	Study Date	Study Description	Patients ID
	CT/PET Data Set	2012-03-26 14:10		10
	Series Description	**Series Date**	**Modality**	**Model name**
	pre-p	2012-03-26 15:27	CT	Nano PET/CT
	pre-p	2012-03-26 15:27	PT	Nano PET/CT
	un-reg	2012-03-26 15:27	PT	Nano PET/CT
	Test Tumor	2011-06-09 14:54		Test Tumor
	Series Description	**Series Date**	**Modality**	**Model name**
	Emission acquisition	2011-06-09 14:54	PT	Inveon MM Platfor

[Browser /customers/inviCRO/2012/WholeBodyAtlas] (open) | Filter: | Type:

WholeBodyAtlas
Status: ROIs Complete
Study Description: Developing a whole body atlas for mouse PET/CT.
Deliverables: Whole Body Atlas
ROI List:brain, thyroid, kidneys, liver, heart, bladder
Voxel Size: 0.2
Due Dates: flexible
Analyst Assignments: placing ROIs- Sydney, TJ. Developing protocol & testing: Nadim. Study management: Kate.
Original Data Location: /customers/invicro/2012/WholeBodyAtlas
Processed Data Location: /customers/invicro/2012/WholeBodyAtlas
Results Directory: /customers/invicro/2012/WholeBodyAtlas

Notes:

/projects/customers/inviCRO/2012/WholeBodyAtlas

Data are located here: https://invicro.ipacs.invicro.com/browser?project=1000

Pre-processed data should be saved here: https://invicro.ipacs.invicro.com/browser?project=1001

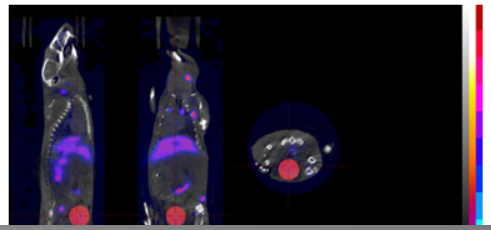
Phantoms located here: \\invicroshare\2012\SPECTCT\phantoms

Voxel Size = 0.2 x 0.2 mm

1. Load the SPECT reconstruction and the CT.
2. In the pre-processing tool, select the following:
   a. Co-register (Fast)
   b. Resample (0.2 mm)
   c. NM/PT Convert unit (uCi)
3. Double-check the registration and adjust if necessary in the reorientation operator.
4. Crop excess blank space if necessary.
5. Remove the bed using Quantification ++.
6. Store the Results to the _p directory.

ROI fitting:

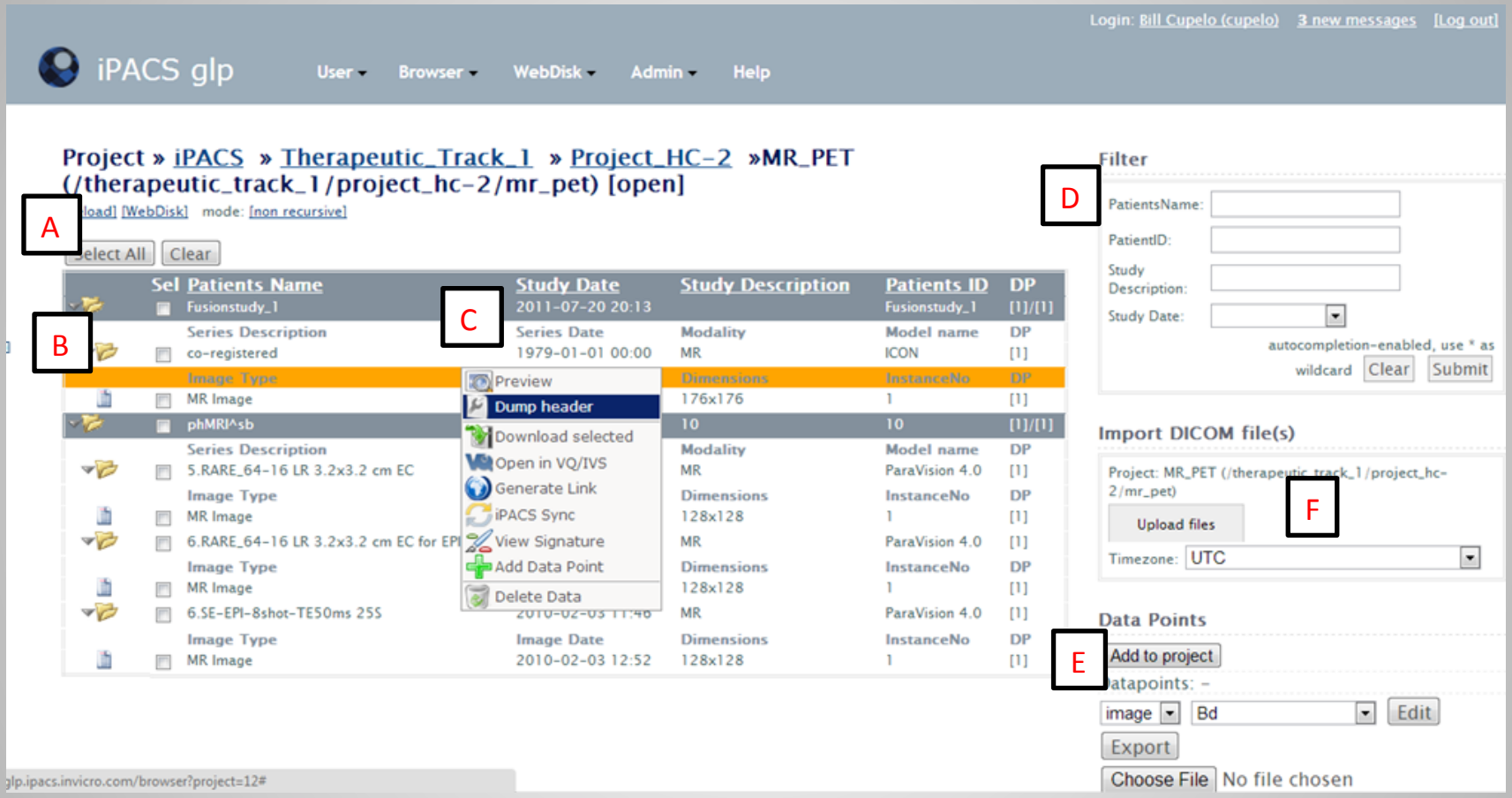
1. Load the SPECT and CT from the _p.
2. Load the ROIs using Import Multiple Images.
3. Fit the ROIs to the data in the Reorientation tool, with the interpolator set on "Nearest NB".
   Example fits:
   (Bladder)



Define roles, deliverables, timelines, basic information for each study.

Image Analysis

Gathering and indexing the metadata

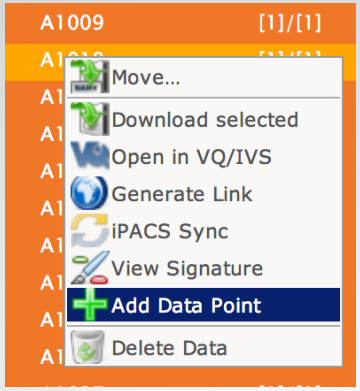


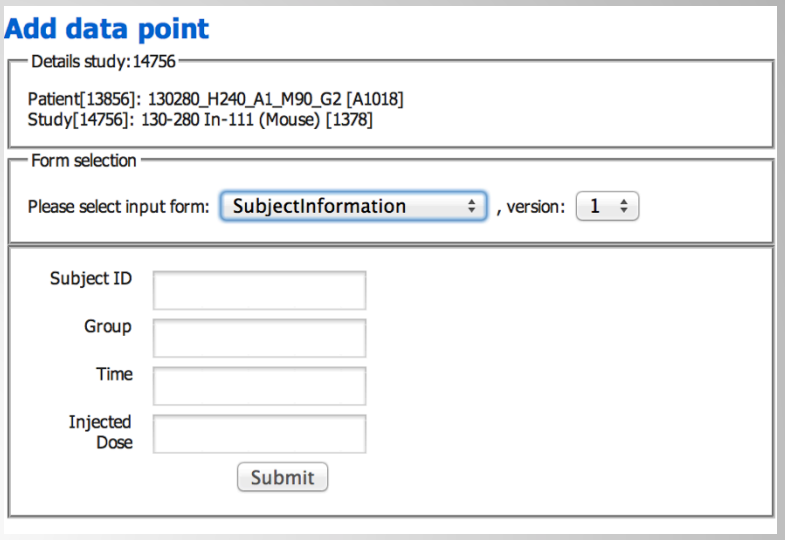
A. Quickly switch between Browser and WebDisk view of a project, and move within project tree
B. The iPACS Browser follows the DICOM standard hierarchy (Patient/Study/Series/Image).
C. Download image data and/or open directly in the VivoQuant™ desktop client.
D. Filter over metadata fields.
E. View data points associated with an individual image (e.g. Injected Dose, Cohort/Group Name, Weight, etc.).
F. Upload DICOM files to current project via web, or use StoreSCU from your DICOM client

Image Analysis

Example: Standard metadata

For an individual





At different levels

2012-03-01 20:49	cupelo	StudyUnits:1	injdoseunit=uCi outputunit=uCi submit=Submit timeunit=d

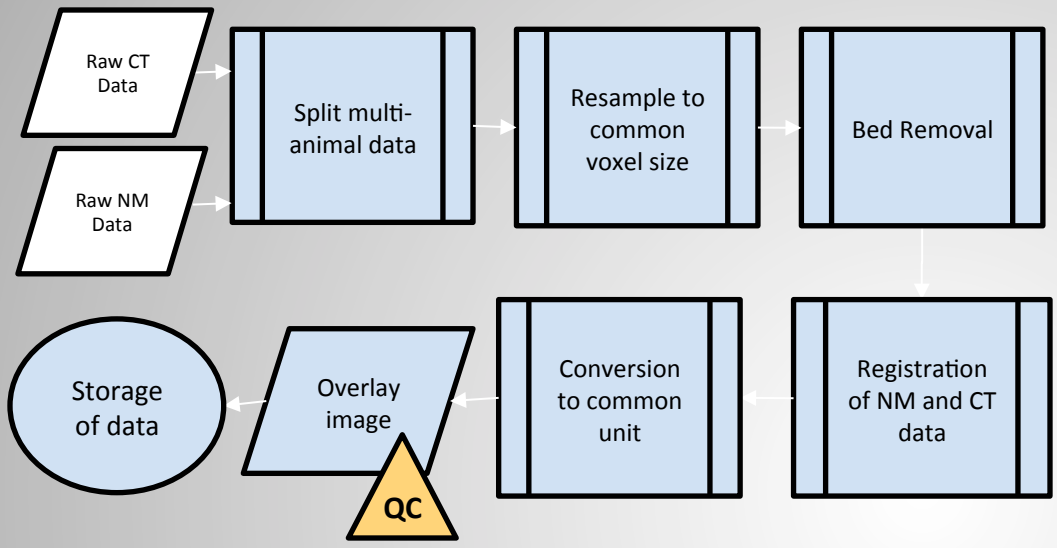
In Batch

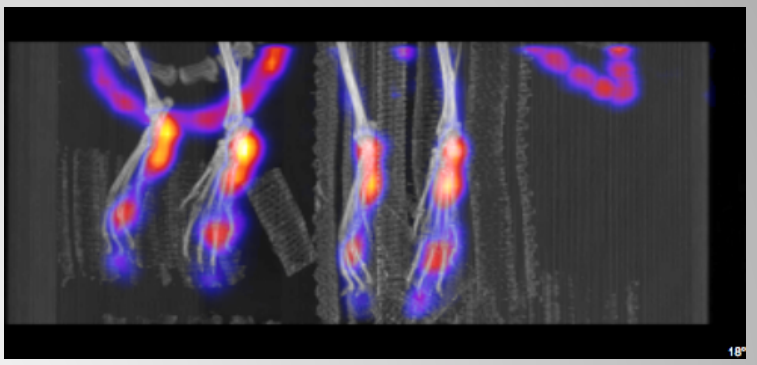
			Subject ID	Group	Timepoint	Injected Dose
130280_H0_A4_M13_G2 (A1018) 2012-08-14T18:44:16	NM	Whole Body SPECT (2) HiSPECT Reconstrution [Co-Reg] 2012-08-14T21:31:45 | 195043.532	1018	2	0.5	1354.53223991579
130280_H0_A4_M13_G2 (A1018) 2012-08-14T18:44:16	NM	Whole Body SPECT (3) HiSPECT Reconstrution [Co-Reg] 2012-08-14T21:31:48 | 200844.918	1018	2	0.75	1350.34337517629
130280_H0_A4_M13_G2 (A1018) 2012-08-14T18:44:16	NM	Whole Body SPECT (4) HiSPECT Reconstrution [Co-Reg] 2012-08-14T21:31:51 | 202646.405	1018	2	1	1346.1636073976
130280_H0_A4_M13_G2 (A1018) 2012-08-14T18:44:16	NM	Whole Body SPECT HiSPECT Reconstrution [Co-Reg] 2012-08-14T21:31:38 | 193224.241	1018	2	0.25	1358.80417523604

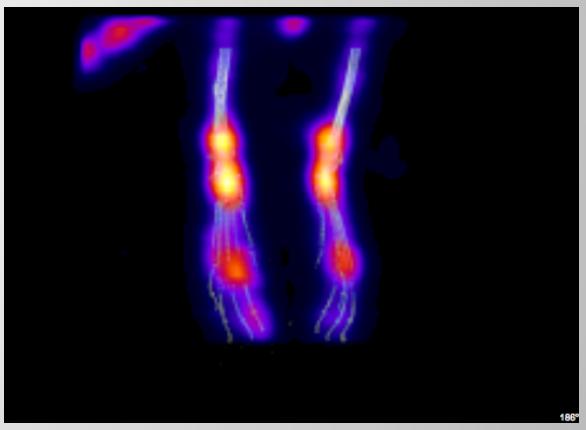
All SPECT (and PET) studies require knowledge of subject ID, group, timepoint, and injected dose.

Image Analysis

Preprocessing Image Data







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  (fffe,e000) na (Item with undefined length #=6)         # u/l, 1 Item
    (0008,0023) DA [20120814]                               #   8, 1 ContentDate
    (0008,0033) TM [160729.832]                             #  10, 1 ContentTime
    (0008,1070) PN [local:Nadim]                            #  12, 1 OperatorsName
    (0008,1155) UI [1.3.6.1.4.1.33793.1.4.1525403793.1824.1343172935.12] #  52, 1 ReferencedSOPInstanceUID
    (0008,2111) ST [SplitROI2DICOM: ROI #1]                 #  22, 1 DerivationDescription
    (0018,1020) LO [VivoQuant 1.22alpha22]                  #  22, 1 SoftwareVersions
  (fffe,e00d) na (ItemDelimitationItem)                   #   0, 0 ItemDelimitationItem
  (fffe,e000) na (Item with undefined length #=6)         # u/l, 1 Item
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    (0008,0033) TM [111330.183]                             #  10, 1 ContentTime
    (0008,1070) PN [local:Whitney]                          #  14, 1 OperatorsName
    (0008,1155) UI [1.3.6.1.4.1.33793.1.4.3613412403.6256.1344974879.28] #  52, 1 ReferencedSOPInstanceUID
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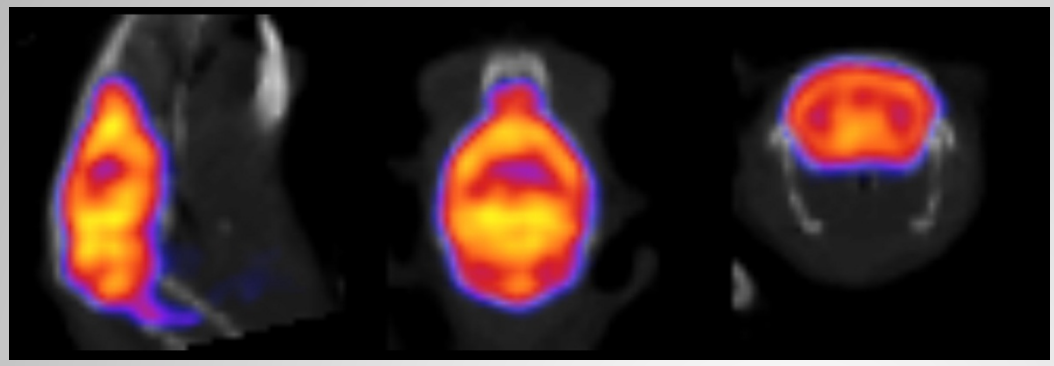
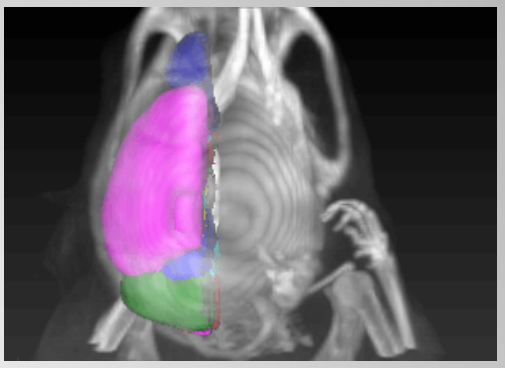
Critical components include:
Units
Documentation

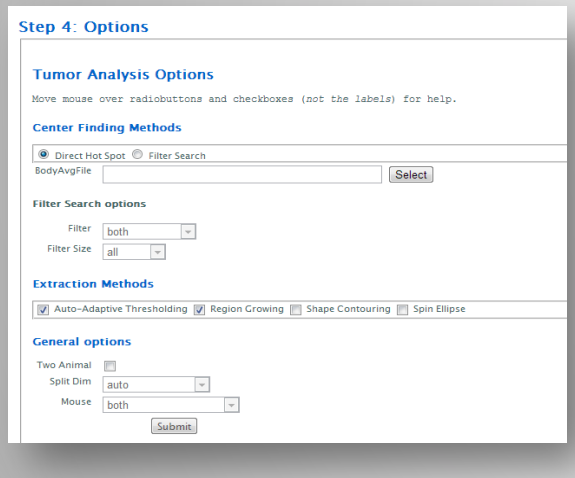
Image Analysis

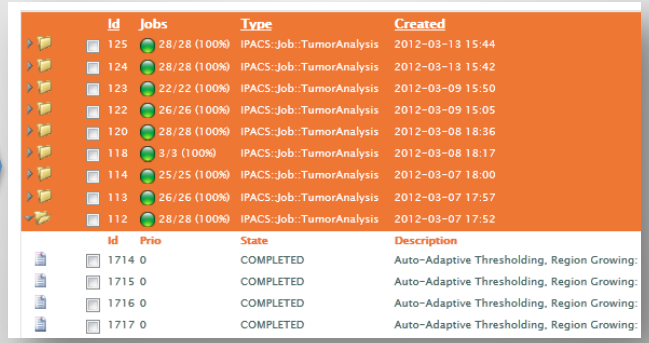
Extracting image information

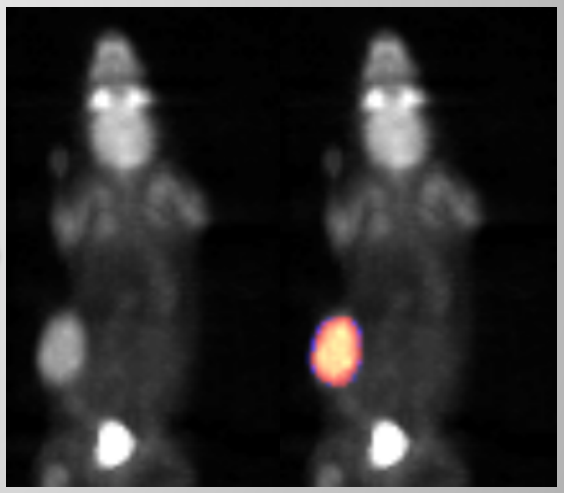




Analysis of radiotracer distribution in the brain







Analysis of radiotracer distribution in xenograft tumors.

Automation, and particularly semi-automation, are capable of reducing observer variability and speeding analysis times, but generalized tools are often time-consuming and expensive to develop.

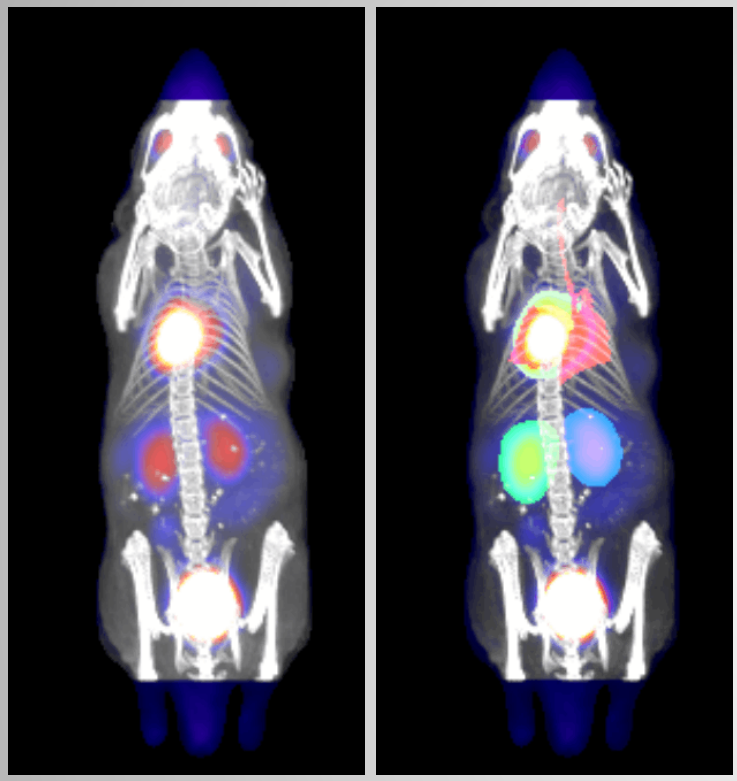
Image Analysis

Extracting Image Information

Standardizing protocols, improving low-level functionality, and removing inefficiencies can be more time-effective and beneficial than “magic segmentation buttons”.

Image Analysis

Storing Extracted Image Information



Sel	Patients Name	Study Date	Study Description	Patients ID	DP
	M1	2012-06-28 20:16	Training	1	-/[1]
	Series Description	**Series Date**	**Modality**	**Model name**	**DP**
	Training	2012-06-28 21:06	CT	ModelName	[1]
	Training	2012-06-28 21:06	NM	ModelName	[1]
	Image Type	**Image Date**	**Dimensions**	**InstanceNo**	**DP**
	NM Reconstruction	2012-06-28 21:09	142x142	1	[15]

Data points for Image[972932]: ORIGINAL\PRIMARY\RECON TOMO\EMISSION []

8 datapoints found

Hierarchy: »series:46173 »studies:15083 »patients:13973 »projects:642 »projects:641 »projects:640 »projects:90 »projects:1 | Include parent points

Filter: Type: , User: Data:

Id	Timestamp	Creator	Form	Data	Command
2570769	2012-09-24 16:02	tliguori	quantification:2	center=[67 109 134] color=darkred concentration=0.0135956 filename=/projects/customers/inviCRO/2012/WholeBodyAtlas/roi/roi-tliguori-M12-1348502471.rmha max=0.000149351 mean=0.000108765 min=8.27276e-05 name=Thyroid roiid=7 setid={7944c342-7639-42ec-93ce-eab7d0e02056} stddev=1.33151e-05 sum=0.130736 unit=µCi volume=9.616 voxels=1202	[Edit] [Delete]
	2012-09-24 16:02	tliguori	quantification:2	center=[63 100 364] color=yellow concentration=0.0244056 filename=/projects/customers/inviCRO/2012/WholeBodyAtlas/roi/roi-tliguori-M12-1348502471.rmha max=0.000288118 mean=0.000195245	[Edit] [Delete]

Critical components include:

Quality Control

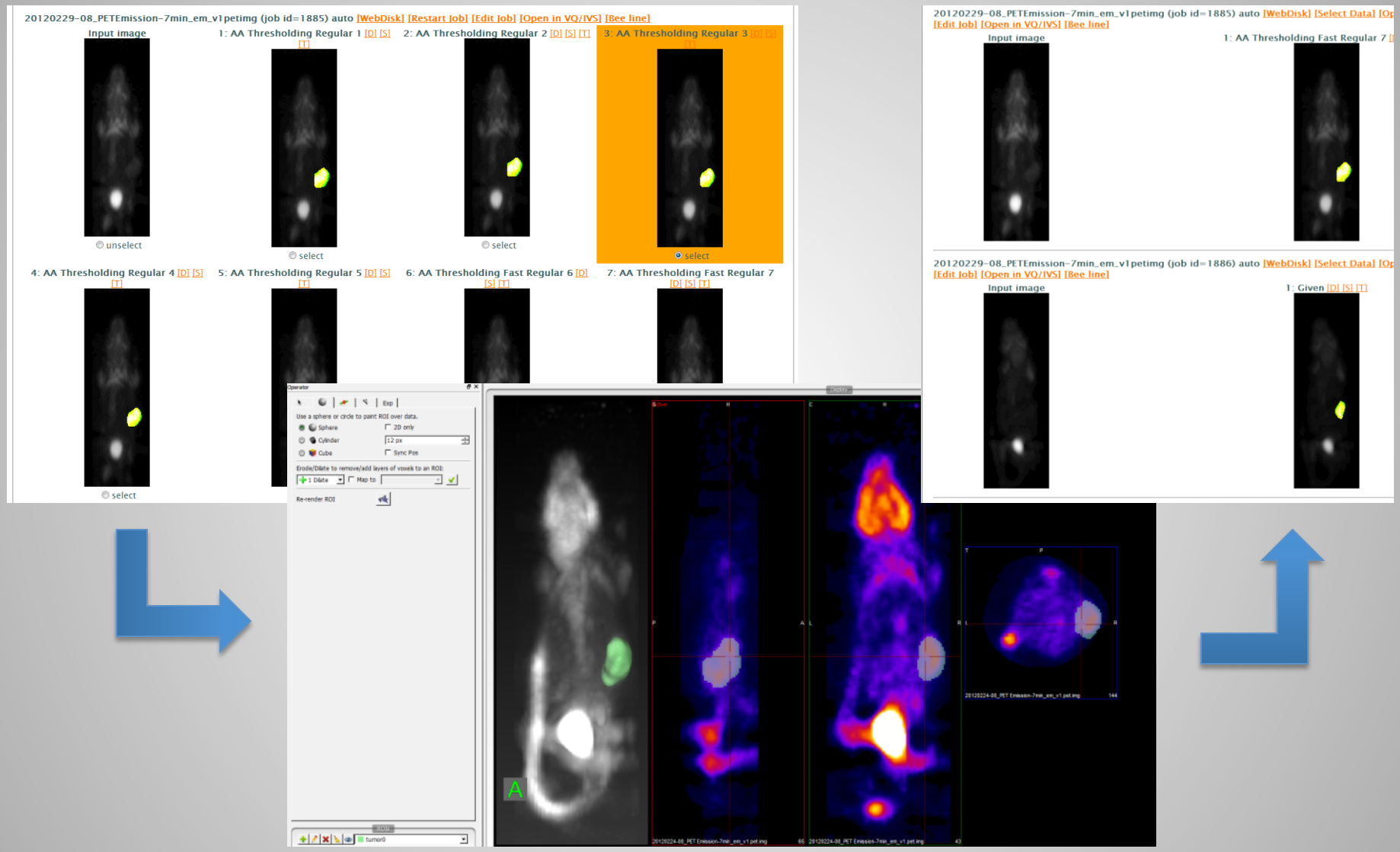
Manual Intervention

Documentation

Standardization

Image Analysis

Editing and Reviewing Regions of Interest

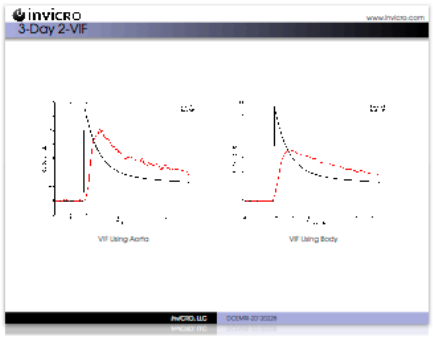


Example: Individual tumors are QC'd, manually edited in VQ, and re-run as necessary.

Image Analysis

Quality Control Records – Qualitative and Quantitative

1



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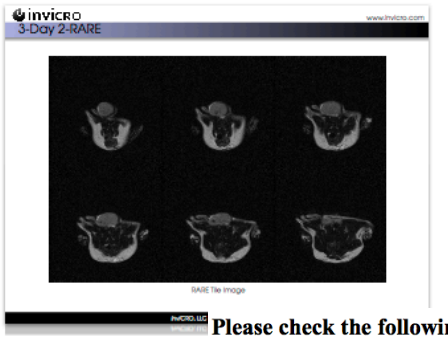

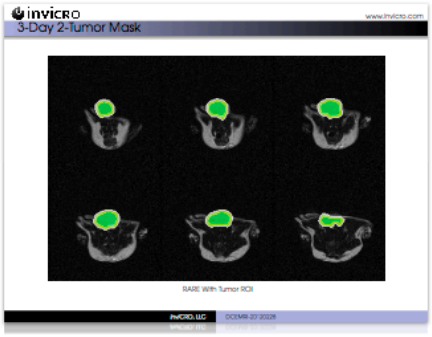

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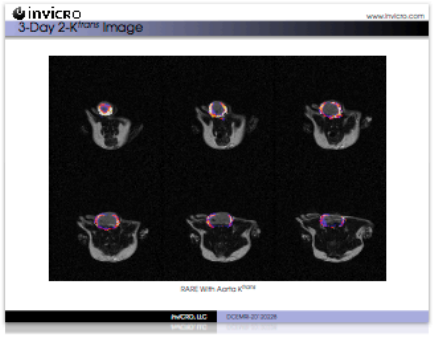
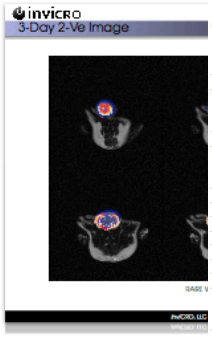


5



Please check the following data:

Cerebellum Volume for ID 1003 in Group Drug A at time 72 Hour
Cerebellum Volume for ID 1005 in Group Drug A at time 24 Hour

CorpusCallosum Volume for ID 1009 in Group Drug B at time 24 Hour

Cortex Volume for ID 1004 in Group Drug A at time 24 Hour

Hippocampus Volume for ID 1005 in Group Drug A at time 24 Hour

Hypothalamus Volume for ID 1001 in Group Drug A at time 72 Hour

Medulla Volume for ID 1005 in Group Drug A at time 24 Hour
Medulla Uptake for ID 1010 in Group Drug B at time 24 Hour
Medulla Conc for ID 1010 in Group Drug B at time 24 Hour
Medulla Conc for ID 1010 in Group Drug B at time 72 Hour

Midbrain Volume for ID 1008 in Group Ctl A at time 24 Hour

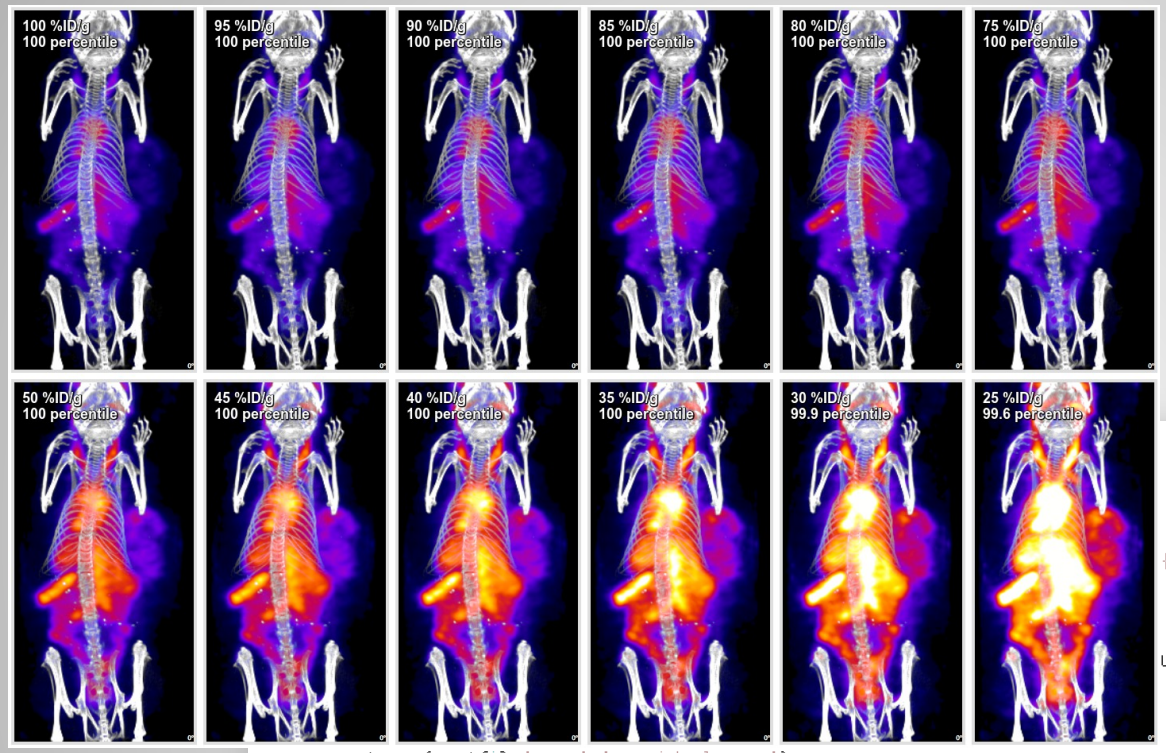
Olfactory Volume for ID 1003 in Group Drug A at time 72 Hour
Olfactory Volume for ID 1005 in Group Drug A at time 24 Hour

Pons Volume for ID 1008 in Group Ctl A at time 72 Hour
Pons Uptake for ID 1010 in Group Drug B at time 24 Hour
Pons Conc for ID 1010 in Group Drug B at time 24 Hour

Quality control of registrations, estimates, segmentations, etc. are essential.

Image Analysis

Extracting image information



Critical components include:
Standardization Units
Standardization Naming
Standardization Storage
Standardization Output

```
{}\n\\begin{figure}[ht]\n'];

um 'cm]{'...

name = strrep(nset{i},'.png','-residuals.png');
tmptext = [tmptext '\\subfigure[' imgcaption ']{\n\\includegraphics[height=' wnum 'cm]{'...
           name '}\n}\n'];

tmptext = [tmptext '\\end{figure}\n\\end{frame}\n\\clearpage\n'];
imgtext{i} = tmptext;
end
```

```
together, go in order

]].SeriesInstanceUID);
es[j].SeriesInstanceUID);

we don't use it.

matching CT
    var pet = VQ.downloadImages(dcmRep, studies[i].StudyInstanceUID, series[0].SeriesInstanceUID);
var ct = VQ.downloadImages(dcmRep, studies[i+3].StudyInstanceUID, series[0].SeriesInstanceUID);
takeImage(dm, ct, pet);
studies[i+3].PatientsName = ''; //Delete the CT image in the list of studies.
}
}
```

Scripting is a powerful tool for accessing image data, image processing, generation of quality control images and spreadsheets.

Image Analysis

Master Spreadsheet and Plotting Tool

```
[%- prj = Model.table('projects').find(planid);
    UNLESS formname.defined; formname = 'quantification'; END;
    UNLESS level.defined; level = 'images'; END;
    UNLESS organs.size;
      organs = prj.findUniqueDPValues('name', formname, level, 1);
    END;
    UNLESS isotope.defined; isotope = '.?'; END;
    UNLESS modality.defined; modality = 'NM'; END;
    unitInfo = prj.lastDataPointsByForm_rs('StudyUnits');
    CSV.push('iPACS Master Spreadsheet');
    CSV.push('Project', prj.toString, '', 'Modality', modality);
    CSV.add('Subject ID','Group',
            'Time (' _ unitInfo.value('timeunit') _ ')',
            'Injected Dose (' _ unitInfo.value('injdoseunit') _ ')');
    IF metadata.size; CSV.add(metadata); END;   # Add extra metadata column headers
    CSV.add('SeriesDesc');
```

/projects/customers/inviCRO/2012/WholeBodyAtlas/extractEnergyWindowNameV

```
[% MODE=csv %]
[%- prj = Model.table('projects').find(474) -%]
[% prj.toString %],,,,,
Series Description,Series Instance UID,SOP Instance UID,Isotope
[% FOR p IN prj.patients -%]
 [% FOR s IN p.studies -%]
  [% FOR t IN s.series -%]
   [% IF t.modality == 'PT' || t.modality == 'NM' -%]
     [% tInfo = t.dataPointsByForms('metadata') %]
       [% FOR i IN t.images -%]
     "[% t.seriesdescription %]",[% t.seriesinstanceuid %],[% i.sopinstanceuid %],[% tInf
     [%- END %]
   [%- END %]
  [%- END %]
 [%- END %]
[%- END %]
```

Subject ID	Group	Time (d)	Inj Dose (uCi)	Tumor Volume (mm^3)	Tumor Uptake (uCi)	Tumor Conc (uCi/mm^3)	Tumor Percent ID (% ID)	Tumor Percent ID/g (%ID/g)
A1	Cohort A	0	206.065	130.792	1.47264	0.0112594	0.714648	5.464
A2	Cohort A	0	210.739	201.368	2.11727	0.0105144	1.00469	4.98932
A3	Cohort A	0	209.5	196.288	2.22335	0.011327	1.06126	5.40667
A4	Cohort A	0	180.346	151.648	1.31912	0.00869853	0.731439	4.82327
A5	Cohort A	0	195.196	180.984	1.91043	0.0105558	0.978722	5.40778
A6	Cohort A	0	184.387	162.192	1.5087	0.00930197	0.818223	5.04478
A7	Cohort A	0	211.943	104.664	1.25813	0.0120206	0.593617	5.67165
A8	Cohort A	0	211.09	138.304	1.1372	0.00822248	0.538727	3.89524
A9	Cohort A	0	197.547	135.36	1.5127	0.0111754	0.765743	5.65708
B1	Cohort B	0	207.49	174.736	2.4013	0.0137424	1.15731	6.62318
B2	Cohort B	0	202.777	293.768	2.48691	0.00846555	1.22643	4.17481
B3	Cohort B	0	220.63	194.784	2.00484	0.0102926	0.908688	4.6651
B4	Cohort B	0	178.072	305.536	1.80093	0.00589435	1.01135	3.31008
B5	Cohort B	0	183.821	176.824	1.58014	0.00893621	0.85961	4.86139
B6	Cohort B	0	210.654	195.184	1.97782	0.0101331	0.938897	4.81032
B7	Cohort B	0	209.055	174.792	1.77007	0.0101267	0.846701	4.84405
B8	Cohort B	0	206.634	184.816	1.36791	0.00740149	0.661996	3.58192
B9	Cohort B	0	207.759	236.504	1.61074	0.00681063	0.775294	3.27814
A1	Cohort A	7	202.265	477.776	1.81976	0.00380882	0.899689	1.88308
A2	Cohort A	7	207.542	1136.36	3.08062	0.00271096	1.48434	1.30622
A3	Cohort A	7	193.35	614.44	3.82172	0.00621984	1.97658	3.21688
A4	Cohort A	7	203.159	279.192	1.55026	0.00555267	0.763079	2.73317

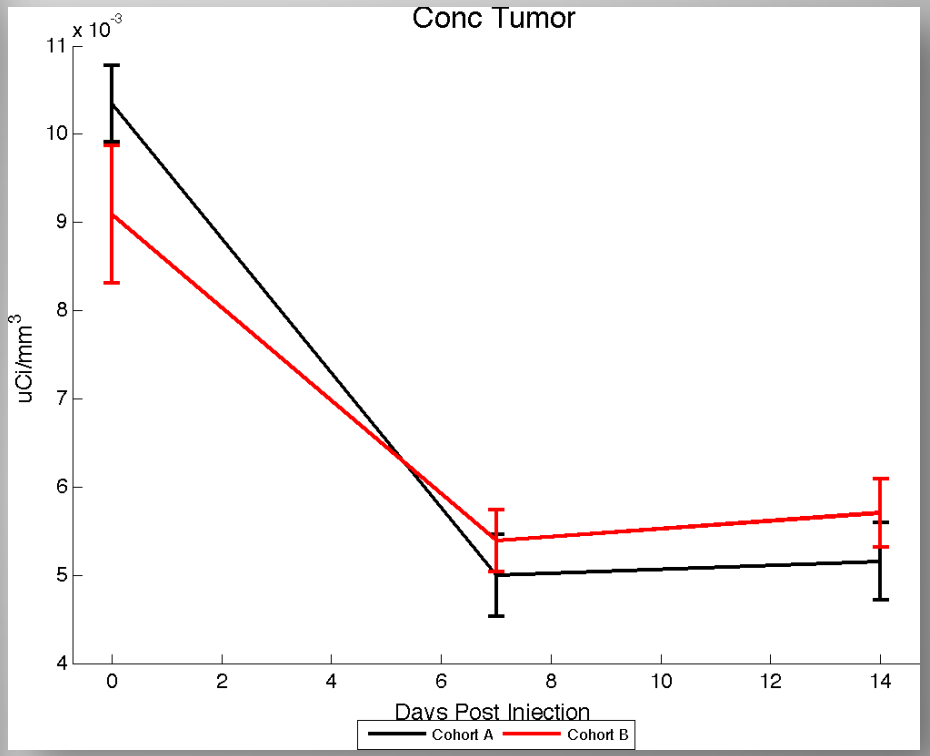
Scripts may be run on the iPACS, in VQ, or locally (using iPACS APIs) to access image information.

Image Analysis

Master Spreadsheet and Plotting Tool

master-summary.txt — Edited

```
Identified 2 Treatment Group(s):
1: Cohort A
2: Cohort B

Subjects in each group:
Cohort A: A1, A2, A3, A4, A5, A6, A7, A8, A9
Cohort B: B1, B2, B3, B4, B5, B6, B7, B8, B9

Time point information for each group:
Units: Day
Cohort A: 0, 7, 14
Cohort B: 0, 7, 14

Injected dose information was found.

Column information:
1: Volume with units mm^3
2: Uptake with units uCi
3: Conc with units uCi/mm^3

Reference organ:
1. Tumor

Other organs:
2. Heart
3. Muscle
```

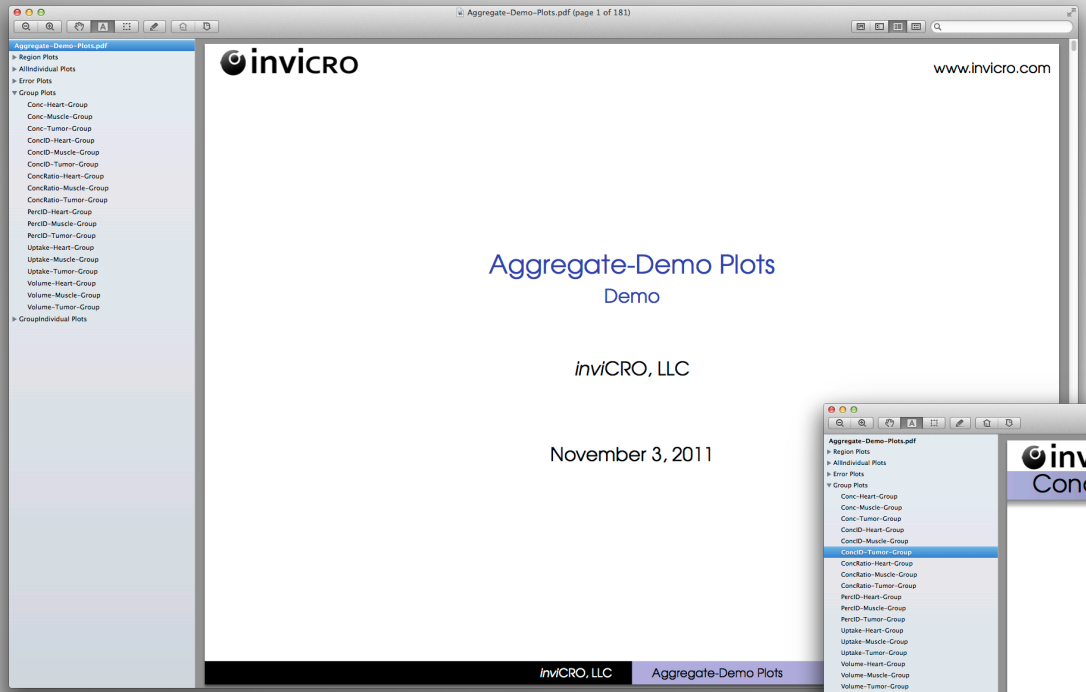


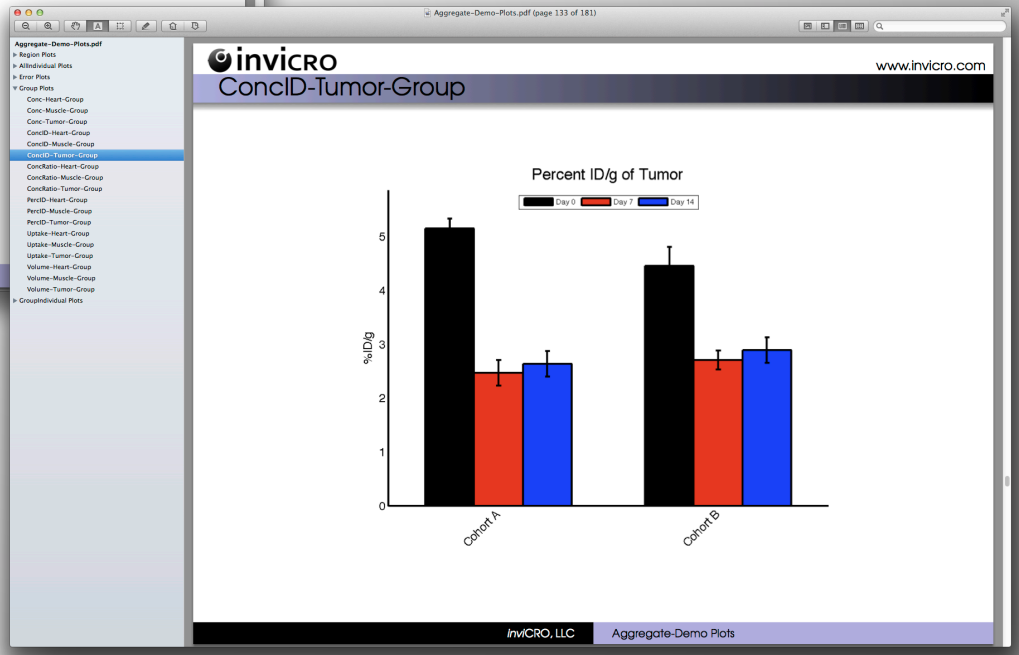
Quantity	Organ	Group	Sample 1	Sample 2	Paired t-test	P-Value	Result	Comment
Volume	Tumor	All Subjects	Day 0	Day 7	Two-tailed	6.77E-08	Mean B > Mean A	VHS
Volume	Tumor	All Subjects	Day 0	Day 14	Two-tailed	1.57E-06	Mean B > Mean A	VHS
Volume	Tumor	All Subjects	Day 7	Day 14	Two-tailed	0.0115557	Mean B > Mean A	S

The plotting tool accepts a settings file and the master spreadsheet to generate output materials, including plots, a summary, output spreadsheets, and hypothesis testing results.

Image Analysis

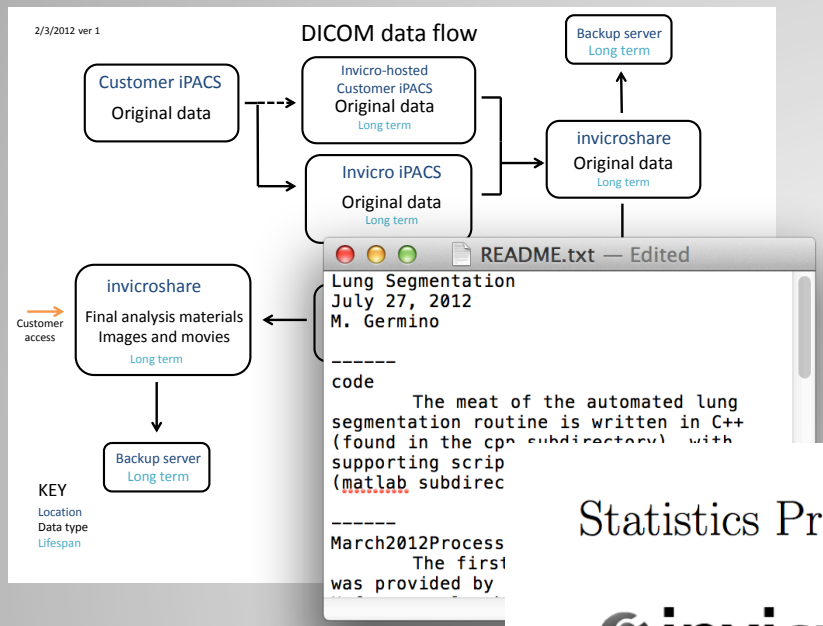
Master Spreadsheet and Plotting Tool





The plotting tool accepts a settings file and the master spreadsheet to generate output materials, including plots, a summary, output spreadsheets, and hypothesis testing results.

Image Analysis
Reporting



README.txt — Edited

```
Lung Segmentation
July 27, 2012
M. Germino

------
code
        The meat of the automated lung
segmentation routine is written in C++
(found in the cpp subdirectory), with
supporting scrip
(matlab subdirec

------
March2012Process
        The first
was provided by
```

Maintaining SOPs and internal documentation.

Statistics Primer

invicro

Au

THE INVICRO COMPREHENSIVE GUIDE TO IMAGE AND MOVIE GENERATION

INVICRO, LLC

Calculating nanoMolar Quantification Factor

Kevin Magalhaes
inviCRO, LLC

July 9, 2012

Critical components include:
Present Succinctly
Maintain Internal Documentation
Standardize Output Structures
Make Everything Available

3.2.2 Functional Connectivity Analysis

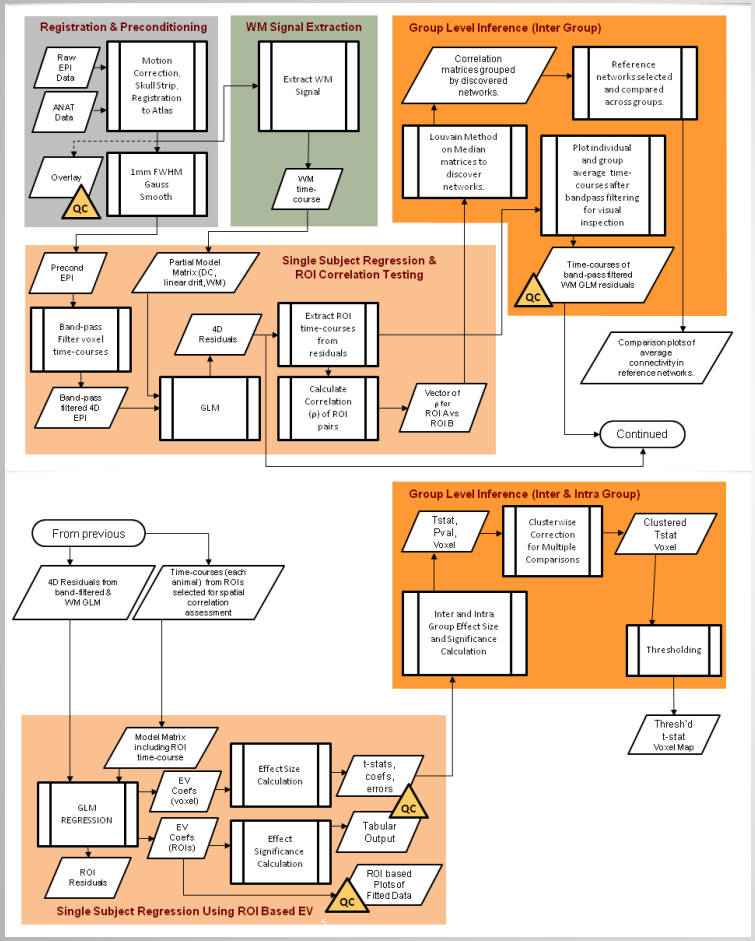


Figure 3: Pipeline for Functional Connectivity Analysis

6 SUPPLEMENTAL

Often, output materials are generated in preparation or during an imaging study. For reference, links to many of these materials are provided here, with a brief description.

Functional connectivity analysis raw materials for subject level analysis using bandpass filter of $0.001Hz$ to $0.01Hz$: Subject output for alternate BPF window

Slides from early considerations of design matrix setup, including use of a longer ideal ramp function for phMRI analysis and example signal fits: Slide Deck 1

Slides from initial examination of effect from cluster-wise correction for multiple comparisons: Slide Deck 2

Slides from initial work on correlation matrix functional connectivity analysis using paired tests: Slide Deck 3

Slides from initial work on GLM based functional connectivity analysis without White Matter regression pre-processing: Slide Deck 4

Slides from initial work testing the effects of bandpass filtering on functional connectivity analysis: Slide Deck 5

Slides comparing results using different registration and slice selection methods for spatial normalization and group analysis: Slide Deck 6

Slides comparing results using the different registrations mentioned above, but with maps that are uncorrected for multiple comparisons. Slide Deck 7

Slides from initial results of phMRI analysis results including workflow at that stage of analysis: Slide Deck 8

Slides comparing results from using White Matter and White Matter plus CSF region signals as nuisance regressors: Slide Deck 9

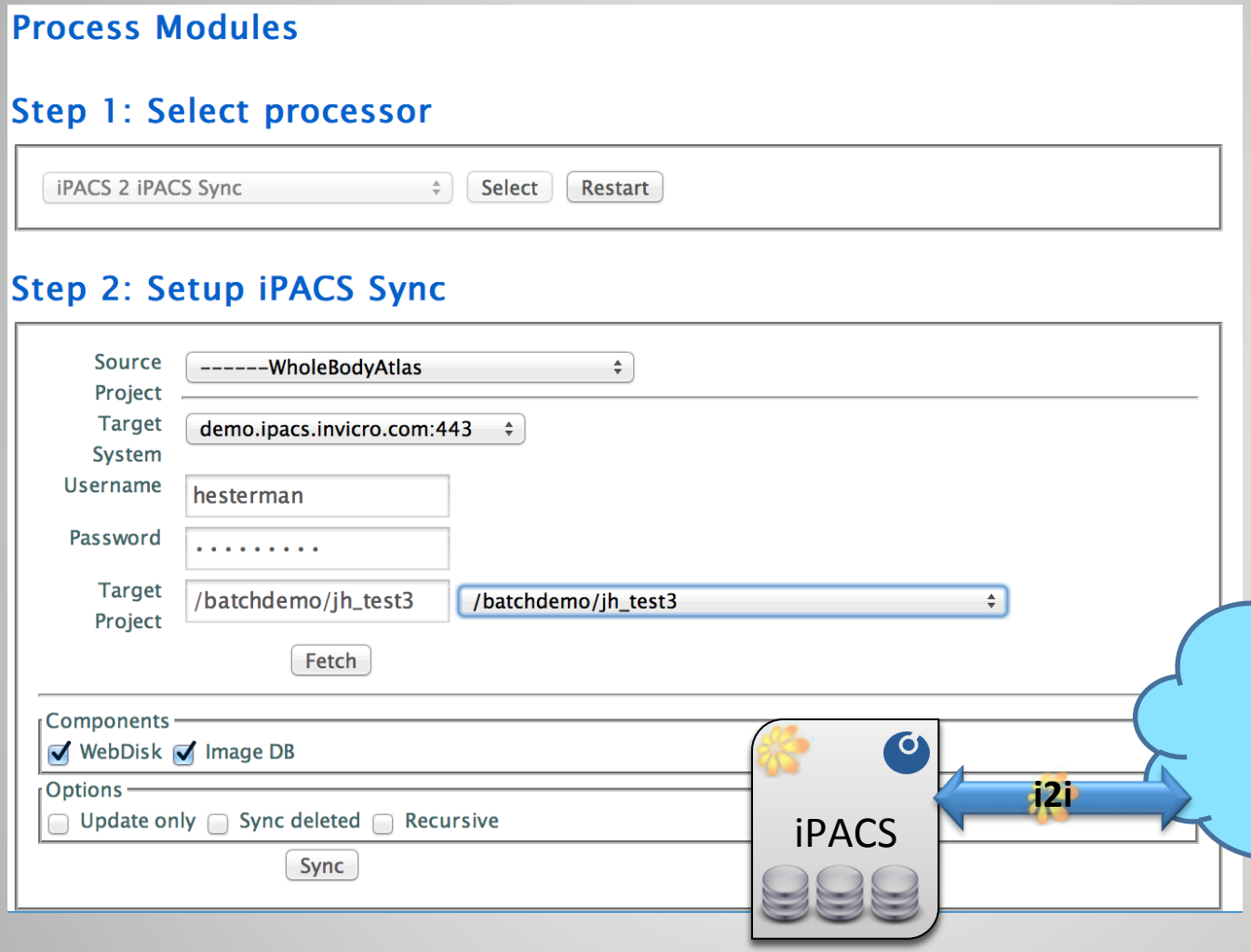
Slides with initial proposal of phMRI and functional connectivity analysis workflows: Slide Deck 10

Slides for results prior to fixing degrees of freedom issue in group analyses.

- phMRI Results: Slide Deck 11
- Un-thresholded Functional Connectivity Results: Slide Deck 12
- MC-Corrected Functional Connectivity Results: Slide Deck 13

Image Analysis

Reporting



Disseminating results entails similar challenges to gathering of data. Methods are required that enable transfer of processed data, metadata, and results (reports, plots, etc.).

Summary

Design, Management, Analysis, Reporting

- Careful planning, including selection of isotope, animal number and kind, mass dose, and radiochemistry strategy are required.
- A variety of pre-imaging assays (i.e., dose escalation studies) can greatly aid the success of the final imaging study.
- Multiple moving, time-sensitive parts require coordination so logistics are critical.
- Clearly defined roles & responsibilities and teamwork are essential to hitting imaging time points successfully.
- Data analyses starts (flow of data from acquisition site to analysis platform) and ends (flow of data from analysis/reporting platform to sponsor) with data management.
- Reproducibility, efficiency, and attention to detail (i.e., units, documented and reproducible computation) are all critical.
- Storage of data and metadata in a generalized, script-accessible, well-documented format important for long-term access and analysis.

Summary

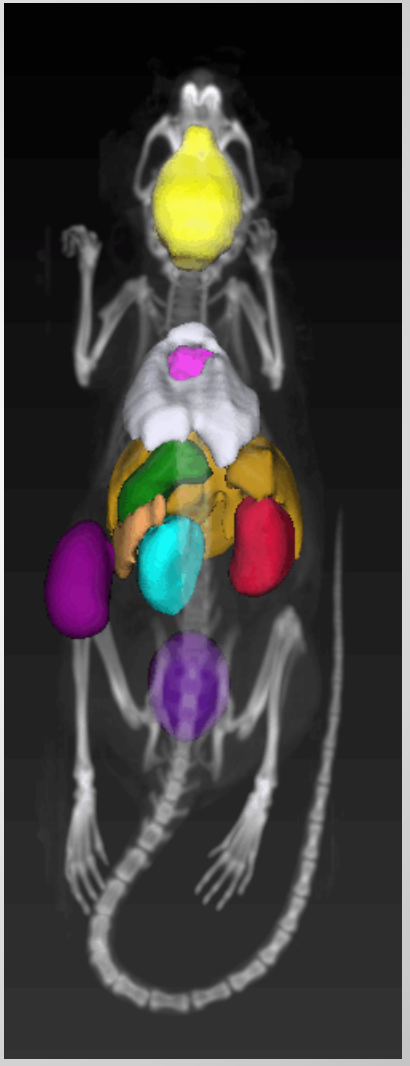
Other Considerations

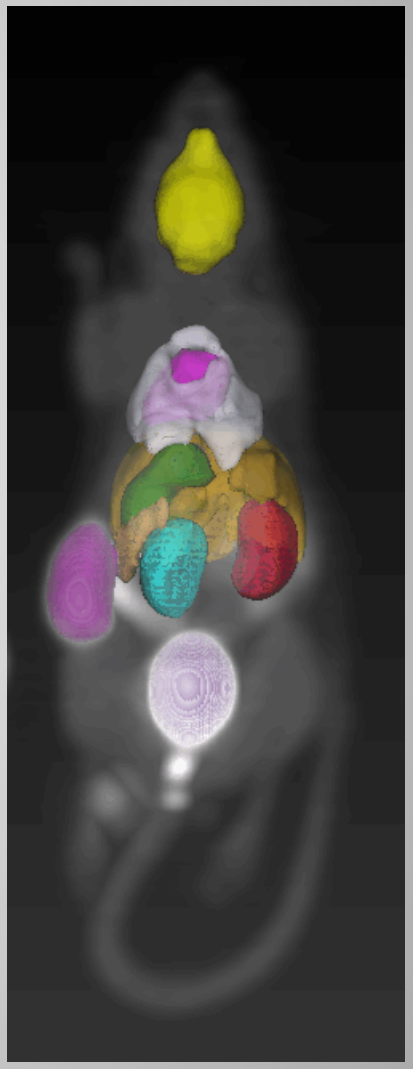
- PK modeling and sub-organ analyses are critical unique features of preclinical in vivo imaging.
- Imaging is expensive but contains a wealth of information – more (i.e., data mining, increased standardization of acquisition/reconstruction protocols) is required to make it possible for image information to be shared across studies.
- The use of MR and optical imaging in conjunction with NM imaging is of growing importance.
- The right applications should be targeted for in vivo imaging studies.

Thursday, 1:30-2:30PM Session

Title: Pre-clinical SPECT/CT-Specific Applications and Case Studies

The content will be case studies, particularly with respect to why we use SPECT/CT in translational research.





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inviCRO, LLC

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