An Imaging Roadmap for Biology Education: From Nanoparticles to Whole Organisms

Biological imaging illustrates the importance of the relationship between biological scale and imaging scale, offers new insights into biological structure and function, brings quantification into biology education, and provides ways of advancing nanomedicine, regenerative medicine, and nuclear medicine which contribute to the NIH Roadmap initiatives This nanoimaging, molecular imaging, and medical imaging teaching unit was developed for three, one hour class periods in an introductory course on ways of knowing biology.

Executive Summary for Teachable Unit

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I. Title: An Imaging Roadmap for Biology Education: From Nanoparticles to Whole Organisms

II. Developer: Dan Kelley

III. Learning Goals and Outcomes

A. Learning Goals

Students will understand the importance of biological scale and imaging scale when producing biological images.

Students will understand how imaging provides scientists and physicians ways of knowing the structure and function of biological processes.

Students will understand that imaging is a quantitative tool in biology, which allows them to measure and interpret images across the biological scale.

Students will understand how nanoimaging, molecular imaging, and medical imaging can advance nanomedicine, regenerative medicine, and nuclear medicine and contribute to the goals of the NIH Roadmap.

B. Specific Learning Outcomes

Students will be able to answer questions about biological images at various biological scales which fosters an understanding of the relationship between biological scale and imaging scale and fosters the development of analytical skills.

Students will be able to answer questions about biological images with fluorescent probes or radioactive markers which fosters an understanding of the way imaging provides information about biological structure and function of biological processes.

Students will be able to answer survey questions on the informativeness and usefulness of 2D images, 3D images, and stereolithographic models which fosters the development of evaluation skills.

Students will be able to demonstrate proficiency using NIH Image J software to quantify biological Images and interpret the quantifications which fosters the understanding that biological images are quantifiable and fosters the development of skills in computer use, analysis and synthesis.

Students will be able to know how nanoimages, molecular images and medical images advance nanomedicine, regenerative medicine, and nuclear medicine which fosters an understanding how imaging contributes to the NIH Roadmap initiatives.

IV. Scientific Teaching Themes:

A. Scientific Teaching

With evolving imaging technology, biological imaging misconceptions develop:

(1) Biological science and imaging science are distinct. This is a misconception because these sciences are symbiotic.
(2) Any imaging technique can image any biological specimen. This is a misconception since there is a relationship between biological scale and imaging scale.
(3) Biological images reveal mainly biological structure. This is a misconception since molecular imaging with fluorescent probes, PET imaging with radioactive markers, and fMRI reveal structure and function of biological processes.
(4) Biological imagines are not quantifiable. This is a misconception since computer software programs like NIH Image J can measure biological images.
(5) Biological images do not advance science or medicine. This is a misconception since imaging can advance nanomedicine, molecular medicine, and nuclear medicine, which contribute to the NIH initiatives.

These misconceptions are addressed in our learning goals by showing how biological scale and imaging scale are related, how biological imaging provides ways of knowing biological structure and function, how biological images can be quantified, and how biological images contribute to the NIH Roadmap initiatives.

We use backward design to create this teaching unit. The concepts that are generally considered difficult to understand such as biological scale, ways of knowing biology, quantifying images, and advancing NIH Roadmap initiatives provide a basis for the learning goals. By transferring learning goals from the perspective of the teacher to learning outcomes from the perspective of the student, we are able to delineate measurable criteria for assessment purposes. Course activities are developed with this in mind.

B. Active Learning

When introducing a topic we select interesting nanoimages, molecular images, and medical images so that students can understand the relationship between biological scale and imaging scale. By introducing fluorescent probes, PET images with radioactive markers, and fMRI, students can gain an understanding how images provide ways of knowing biological structure and function. Through introduction of image analysis software, NIH Image J, students are able to extend their conceptual understanding of imaging analysis into a computer skill using real data. In this way they come to understand the concept that biological images can be quantified. Contributions of nanoimaging to nanomedicine, molecular imaging to regenerative medicine, and PET nuclear medical imaging to nuclear medicine can advance NIH Roadmap initiatives.

C. Assessment

Assessments help determine whether or not learning goals and specific learning outcomes have been accomplished. Written answers to questions about biological scale using nanoimages, molecular images, and medical images help foster an understanding of the relationship between biological scale and imaging scale as well as the development of analytical skills.

Written answers to questions about biological structure and function using images with fluorescent probes and radioactive markers help determine an understanding of the way imaging provides ways of knowing biological function and develops analytical skills.

Answers to survey questions about the quality of visual information and biological utility of 2D images, 3D images, and stereolithographic models help develop evaluation skills.

Answers to questions about quantification of images help determine students' proficiency with NIH Image J software.

Pre and post quizzes determine how much knowledge students actually have acquired and how well they have developed new skills. The in-class activities are meant to help students build knowledge and skills.

D. Diversity

Diversity in students' cultural and educational backgrounds is accounted for by incorporating multiple modes of teaching and assessment forms. To minimize discrepancies in education, we review background information in our minilectures. We engage students of diverse cultures by introducing scientists from different nations who have contributed to imaging. Audiovisual aids help clarify difficult material. We use the video, "Power of Ten," to introduce the concept of biological scale and a movie clip of the "Hulk" to illustrate the effect of fluorescence. Using a computer software program, NIH Image J, we quantify images and using hand held models of a brain and Phineas Gage's skull we show how images can be utilized to create stereolithographic models. To assess learning gains we use a variety of assessment forms: oral discussion, written answers, surveys, and pre and post assessments.

E. **Alignment: Schedule of in-class activities that links learning outcomes and activities/assessment**

Biological Images	**Activity/Assessment**	**Learning Outcomes**
Nanoimages	Video, "Powers of Ten"	-Will be able to understand biological scale
	Open Ended Question about biological and imaging scales	-Will be able to understand biological scale's relationship to imaging scale
	Use NIH Image J to quantify height and intensity of Nanobucky	-Will understand that images can be quantified -Will be able to use NIH Image J
	Minilecture: Nanoimages aid nanotechnology to create nanodevices which can advance nanomedicine	-Will understand how nanoimages can contribute to NIH Roadmap initiatives in nanomedicine
Molecular Images	Problem Solving: Questions about the fluorescent rabbit, Alba	-Will understand how fluorescence incorporates on the systemic-level scale -Will be able to develop analytical skill reading fluorescent probes' wavelength charts
	Mini-Demonstration: Use NIH Image J to quantify intensity of DAPI stained nucleus of endothelial cell	-Will understand how fluorescent probes incorporate on the cellular-level scale -Will understand that images can provide ways of knowing biological structure -Will be able to use NIH Image J
	Monitor eGFP human embryonic stem cell differentiation Use NIH Image J to quantify intensity of eGFP stem cells	-Will understand how images can provide ways of knowing biological function -Will be able to use NIH Image J
	Minilecture: eGFP human embryonic stem cell is involved in tissue re-engineering which advances regenerative medicine	-Will understand how fluorescent molecular images can contribute to NIH Roadmap initiatives in regenerative medicine.
Medical Images	Answer Survey: Case Study: CT of Phineas Gage Skull Evaluate 2D image, 3D virtual reality image, and stereolithographic model of skull for visual information	- Will understand how images can be used to create stereolithographic model of Gage's skull - Will be able to develop the skill of evaluation
	Answer Survey: MRI of brain Evaluate 2D image, 3D virtual reality image, and sterolithographic model of brain for visual information	-Will understand how images can be used to create stereolithoraphic model of brain -Will be able to develop the skill of evaluation
	Answer Survey: Evaluate any 2D image, 3D virtual reality image, and sterolithographic	-Will be able to develop skills of evaluation, analysis, and synthesis

	model for informativeness and usefulness	
	Answer PET Questions using PET brain image with radioactive marker	-Will understand how PET images provide ways of knowing structure and function -Will understand how PET images with radioactive marker can contribute to NIH Roadmap initiatives in nuclear medicine
Web Resources	Introduction to Web Resources	-Will understand that the internet has imaging resources
Assessment Forms	Assessment Forms: Oral Discussion Written Questions Surveys Pre and Post Quizzes	-Will be able to assess learning gains from the Teaching Unit

V. Teaching Plan

	Topic	Activity/Assessment	Goals and Learning Outcome
Pre-Day 1	Assessment	Answer Pre-Quiz online	Students and instructors will be able to assess prior knowledge about imaging.
Day 1	**Nanoimaging**		
0-5	Overview	**Minilecture**	Students will understand the major content of TU, the learning goals, the learning outcomes, and how images contribute to the NIH Roadmap initiatives
Elicit/Engage Ask students to think about the contents of the TU and what they want to learn from it.			
5-15	Biological Scale	**Video, "Powers of Ten"**	Students will understand biological scale from nanoscale to systemic-level scale.
Elicit: Ask students to think about what they already know about biological scale			
15-20		**Open Ended Question:** Biological scale and imaging scale	Students will understand the relationship between biological scale and imaging scale.
Elicit/Engage: Ask students to think about the imaging techniques available at different scales and the biological specimens that can be imaged at those scales. This activity addresses the misconception that biological sciences and imaging sciences are distinct and not symbiotic.			
20-35	Nanoimaging	**Minilecture**	Students will understand that the biological scale of nanoparticles is related to the imaging scale of the electron microscope. Students will understand that nanoimages provides ways of knowing biological structure and function in the nanoscale. Students will understand that nanoimaging and nanotechnology can help develop nanodevices, which can advance nanomedicine and contribute to the NIH Roadmap initiatives.
35-55	Nanobucky	**Activity:** Use NIH Image J to quantify height and intensity of Nanobucky.	Students will understand that nanoimages can be quantified using NIH Image J Students will be able to use NIH Image J
Engage/Explore/ Evaluate/Extend: Ask students to work as a group to measure the height and intensity of the electron microscopic image of Nanobucky using NIH Image J software. This will address the misconception that images cannot be quantified. Ask students to think how nanodevices can advance nanomedicine. To guide them, the teacher will present PowerPoint images of nanodevices that are currently being tested.			

Day 2	**Molecular Imaging**		
0-15	Fluorescent Probes	**Minilecture**	Students review the answers for the activity in Day 1. Students will understand that the biological scale of molecules is related to the imaging scale of the light microscope. Students will understand how fluorescent probes in molecular images provide ways of knowing biological structure and function of molecular particles. Students will understand how fluorescence is incorporated into the systemic scale of the rabbit Alba.
Evaluate/ Elaborate: Review answers for Day 1 Activity. Review biological and imaging scales. To illustrate fluorescence the instructor should show a movie clip of "The Hulk"			
15-20	Fluorescence Systemic Scale	**Problem Solving** Answer questions about the fluorescence of the rabbit Alba	Students will understand eGFP and what makes Alba glow green. Students will be able to develop their analytical skill by reading wavelength and color charts of fluorescent probes.
Elicit/Engage/ Evaluate: Ask students to think what causes fluorescence in terms of excitation and emission wavelength. The instructors should provide background information and explain the physics behind wavelength and color of fluorescent probes.			
20-25	Fluorescent Probes Cellular Scale	**Mini-Lecture** Fluorescence can be incorporated into endothelial cells	Students will understand that there are different colored fluorescent probes or stains used in molecular imaging that aid understanding of biological structure.
25-35	Endothelial Cell	**Mini-Demonstration** Use NIH Image J to quantify intensity of DAPI stained nucleus of endothelial cell	Students will understand that molecular imaging can be quantified Students will be able to apply knowledge of NIH Image J
Elicit/Engage/ Evaluate: Ask students to think how different structures in a cell are tagged with different fluorescent probes. Instructor should show structure of fluorescent probes and images of various cells with attached probes.			
35-45	Human Embryonic Stem Cell	**Activity:** Answer questions about differentiation observed in molecular images of human embryonic stem cells tagged with eGFP.	Students will understand that molecular images of human embryonic stem cells tagged with eGFP provide ways of knowing biological function, differentiation Students understand that human embryonic stem cell information can advance tissue re-engineering and contribute to regenerative medicine and the NIH Roadmap initiatives.
Evaluate/ Extend: The instructor should show the different types of human embryonic stem cell differentiation. Ask students to observe the molecular images of stem cells tagged with eGFP to determine the type of differentiation observed. This activity addresses the misconception that molecular images relate structure not function. Ask students to think how information from these molecular images can advance tissue reengineering and improve regenerative medicine.			
45-55	Molecular images	**Discussion** Molecular imaging Fluorescent probe, eGFP	Students will understand that molecular images tagged with fluorescent probes provide ways of knowing biological structure and function
Engage/Explore: Ask students to think about what other cells can be tagged and what other biological functions can be studied.			
Day 3	**Medical Imaging**		
0-10	X ray	**Minilecture**	Students will understand that X-ray images increase our knowledge of biology for example the structure of DNA.

			Students will understand that X ray images are used in diagnosis and crystallography.
Elicit/Engage/ Evaluate: Review biological and imaging scales. Ask students to think about their own exposure to X rays. This minilecture addresses the misconception that images do not advance science and medicine. The instructor should show the crystallographic image of DNA that was used to figure out its structure.			
10-20	CT	**Minilecture:** CT Case Study: Phineas Gage Images and Model of Skull	Students will understand the importance of relating biological scale with the imaging scale of the CT scanner when producing medical images of Gage's skull. Students will understand that medical images of Phineas Gage's skull brought to light the relationship between brain structure and function.
Elict/Engage/ Evaluate: Instructor should tell Phineas Gage's story and show CT images of Gage's skull. Ask students to think about what brain structures were damaged and how that affected Gage's personality. This addresses the misconception that images do not advance science and medicine.			
20-25	Survey	**Activity**: Answer survey questions about Gage's skull images and model.	Students will develop their evaluation skill by considering the visual information obtained from the 2D image, the 3D virtual reality image, and the stereolithographic skull model of Gage
Elict/Engage/ Evaluate: Instructor should show 2D images and 3D virtual reality images of Gage's skull. Instructor should explain how a stereolithographic model of Gage's skull was made from CT images and should pass the skull model around class for evaluation. This addresses the misconception that images do not advance science and medicine.			
25-33	MRI	**Minilecture** MRI fMRI Images and Model of Brain	Students will understand the importance of relating biological scale with the imaging scale of the MRI when producing medical images of a brain. Students will understand that fMRI images of the brain show a relationship between brain structure and function.
33-37	Survey	**Activity:** Answer survey questions about brain images and model. Answer survey questions about images and model in general	Students will develop their evaluation skill by considering the visual information obtained from the 2D image, the 3D virtual reality image, and the stereolithographic brain model Students will develop their evaluation skill by considering the informativeness and usefulness obtained from the 2D image, the 3D virtual reality image, and the stereolithographic models.
Elict/Engage/ Evaluate: Instructor should show 2D images and 3D virtual reality images of a brain. The instructor should explain how a stereolithographic model of the brain was made from MRI images and should pass the brain model around class for evaluation			
37-42	PET	**Minilecture** PET Radioactive Marker: 18-Fluorodeoxyglucose (18FDG)	Students will understand the importance of producing nuclear medical images with 18FDG. a radioactive marker, which traces the location of high glucose metabolism as occurs in a tumor. Students will understand that nuclear medical images can advance nuclear medicine that contributes to the NIH Roadmap initiatives.
42-52	PET	**Activity:** Answer questions using PET brain images with 18 FDG marker	Students will understand that the PET imaging process uses radioactive contrast and provides ways of knowing biological structure and function
Evaluate/Engage/Elaborate: Ask students to think about the importance of using radioactive contrast material in PET images.			

Instructors should show the chemical structure of the radioactive marker. This addresses the concept that imaging is a multidisciplinary science.			
52-54	Web Resources	**Minilecture:**	Students will understand that the internet has imaging resources. Students will be able to develop their computer skills.
54-55	Course Summary	**Course Summary**	Students will understand that imaging is a quantitative tool which provides ways of knowing biology, and advances nanomedicine, regenerative medicine, and nuclear medicine which contribute to the goals of the NIH Roadmap.
Online	Assessment	**Post Quiz Online**	Students and instructors will be able to assess learning gains

VI. TEACHING MATERIALS

A. Schedule

These materials can be used in three, 50-minute class periods.

B. Notes/ Supplementary Materials

Teaching slides are attached.

C. Surveys

Survey: Pre-Quiz

To what extent do you have knowledge in the following areas:

	Not at all	A little	Somewhat	A lot	A great deal
Nanoimaging	☐	☐	☐	☐	☐
Molecular Imaging	☐	☐	☐	☐	☐
System Level Imaging	☐	☐	☐	☐	☐
Imaging as a quantitative tool	☐	☐	☐	☐	☐
Imaging as a non-invasive tool	☐	☐	☐	☐	☐
Biological Scale	☐	☐	☐	☐	☐
Biological Imaging Tools	☐	☐	☐	☐	☐
Using Imaging Software	☐	☐	☐	☐	☐
Imaging Internet Resources	☐	☐	☐	☐	☐

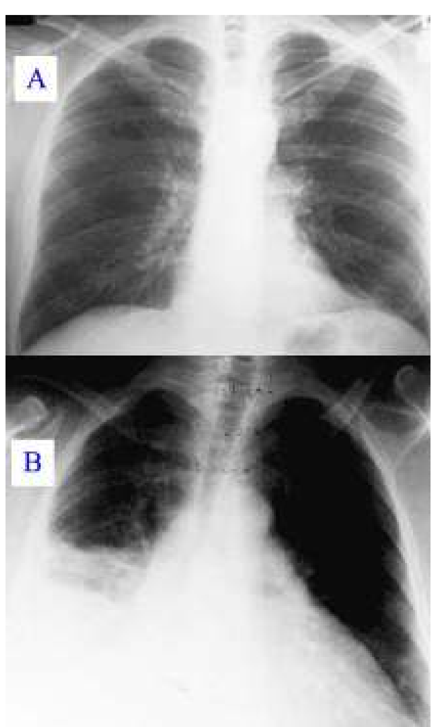
How much skill with imaging do you have in:

	Not at all	A little	Somewhat	A lot	A great deal
Developing Hypotheses from Images	☐	☐	☐	☐	☐
Interpreting Imaging Studies	☐	☐	☐	☐	☐
Recognizing Biological Scale in Images	☐	☐	☐	☐	☐
Quantifying Images	☐	☐	☐	☐	☐

1) Define biological scale and its relation to imaging?

2) Identify 3 imaging techniques and describe how they are used to ask and answer questions about biological processes or disease. Do not use X-rays as an example.

3) Chest X rays of a patient taken at different times show (A) healthy lungs and (B) lungs with inflamed tissue due to pneumonia. Describe the steps you would take to <u>quantify</u> differences between images A and B?



Right Left

http://www.answers.com/topic/pneumonia-x-ray-jpg-1

Survey: Medical Imaging

Student ID ___________________________________

Rate the quality of visual information contained in the following models.

Model	Inferior	Similar/ Equivalent	Superior (similar information more rapidly assimilated)	Superior (additional information provided)
Brain Cortical Surface				
2D	*Baseline*			
VRML	☐	☐	☐	☐
Stereolithograph	☐	☐	☐	☐
Phineas Gage Skull Injury				
2D	*Baseline*			
VRML	☐	☐	☐	☐
Stereolithograph	☐	☐	☐	☐

Evaluate the usefulness of different model types for understanding biology:

	A little	Somewhat	A lot	A great deal
2D	☐	☐	☐	☐
VRML	☐	☐	☐	☐
Stereolithograph	☐	☐	☐	☐

3. Survey: Post-Quiz

Student ID

Do you own a laptop? ☐Yes ☐No

Did you use NIH Image J for class on your own laptop? ☐Yes ☐No

If yes, to what extent do you agree with the following statement:

	Not at all	A little	Somewhat	A lot	A great deal
Image J was easy to get working on my laptop.	☐	☐	☐	☐	☐

Please grade Dan Kelley, the instructor for the imaging unit:

	A	B	C	D	F
Instructor's ability to stimulate interest	☐	☐	☐	☐	☐
Instructor's interest for the subject	☐	☐	☐	☐	☐
Instructor's ability to explain concepts clearly	☐	☐	☐	☐	☐
Instructor's effectiveness	☐	☐	☐	☐	☐
Instructor's enthusiasm for the subject	☐	☐	☐	☐	☐
Overall grade	☐	☐	☐	☐	☐

To what extent did your knowledge increase in the following areas as a result of your work in the imaging unit:

	Not at all	A little	Somewhat	A lot	A great deal
Nanoimaging	☐	☐	☐	☐	☐
Molecular Imaging	☐	☐	☐	☐	☐
System Level Imaging	☐	☐	☐	☐	☐
Imaging as a quantitative tool	☐	☐	☐	☐	☐
Imaging as a non-invasive tool	☐	☐	☐	☐	☐
Biological Scale	☐	☐	☐	☐	☐
Biological Imaging Tools	☐	☐	☐	☐	☐
Using Imaging Software	☐	☐	☐	☐	☐
Imaging Internet Resources	☐	☐	☐	☐	☐
NIH Roadmap	☐	☐	☐	☐	☐

How much did the following help you learn about imaging:

	Not at all	A little	Somewhat	A lot	A great deal
Mini Lectures	☐	☐	☐	☐	☐
Group Activities	☐	☐	☐	☐	☐
Image J	☐	☐	☐	☐	☐
Printed 3D models	☐	☐	☐	☐	☐
Virtual 3D models	☐	☐	☐	☐	☐
Internet Resources	☐	☐	☐	☐	☐
The Topics Covered	☐	☐	☐	☐	☐
Overall Course	☐	☐	☐	☐	☐

To what extent did the imaging unit emphasize that:

	Not at all	A little	Somewhat	A lot	A great deal
Imaging impacts society	☐	☐	☐	☐	☐
Imaging provides a way of knowing biology at different scales	☐	☐	☐	☐	☐
Imaging is an important part of the NIH Roadmap	☐	☐	☐	☐	☐

How much has this unit added to your imaging skills in:

	Not at all	A little	Somewhat	A lot	A great deal
Developing Hypotheses from Images	☐	☐	☐	☐	☐
Interpreting Imaging Studies	☐	☐	☐	☐	☐
Recognizing Biological Scale in Images	☐	☐	☐	☐	☐
Quantifying Images	☐	☐	☐	☐	☐

To what extent did you make improvements in the following as a result of your work in this imaging unit:

	Not at all	A little	Somewhat	A lot	A great deal
Enthusiasm for this field	☐	☐	☐	☐	☐
Interest in pursuing imaging courses	☐	☐	☐	☐	☐
Importance of this field	☐	☐	☐	☐	☐
Confidence in your ability to take part in this field	☐	☐	☐	☐	☐
Understanding that imaging techniques impact society	☐	☐	☐	☐	☐
Understanding that imaging offers new insights into biological structure and function	☐	☐	☐	☐	☐
Understanding that imaging impacts medicine	☐	☐	☐	☐	☐
Understanding that future patients will benefit from ongoing imaging research	☐	☐	☐	☐	☐

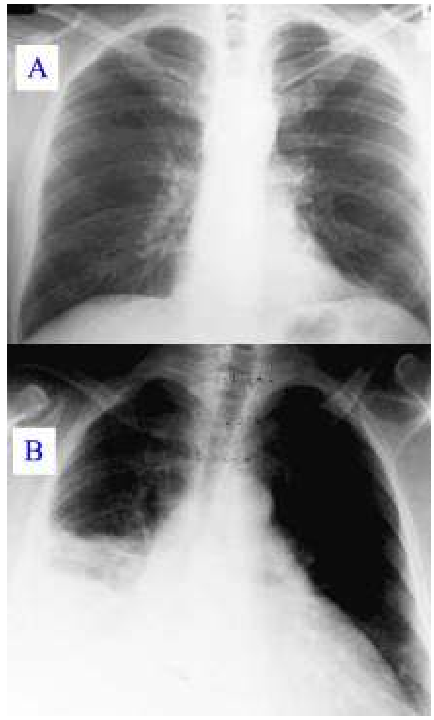
Please answer the extent to which you agree with these statements:

	Not at all	A little	Somewhat	A lot	A great deal
Imaging should be an integral part of biology education	☐	☐	☐	☐	☐
Imaging is an integrated, multidisciplinary field	☐	☐	☐	☐	☐
The overall content of the course was appropriate	☐	☐	☐	☐	☐
Image J contributed positively to this course	☐	☐	☐	☐	☐
After taking this course, I would like to pursue an imaging career	☐	☐	☐	☐	☐
Future imaging courses should use the same format as this course	☐	☐	☐	☐	☐

1) Define biological scale and its relation to imaging?

2) Identify 3 imaging techniques and describe how they are used to ask and answer questions about biological processes or disease. Do <u>NOT</u> use X-rays as an example.

3) These chest X rays of a patient taken at different times show (A) healthy lungs and (B) lungs with inflamed tissue due to pneumonia. The letters A and B are written on the patient's anatomical right side. Describe the steps you would take to quantify differences between images A and B?



http://www.answers.com/topic/pneumonia-x-ray-jpg-1

4) Comments about the imaging unit?

Classroom Resources for Ways of Knowing Biology 2007: Imaging

NIH Videocast
Zerhouni/Lipincott-Scwartz
http://videocast.nih.gov/launch.asp?13093

Electron microscope
http://www.mos.org/sln/sem/seminfo.html

NanoBucky
http://hamers.chem.wisc.edu/research/nanofibers/index2.htm

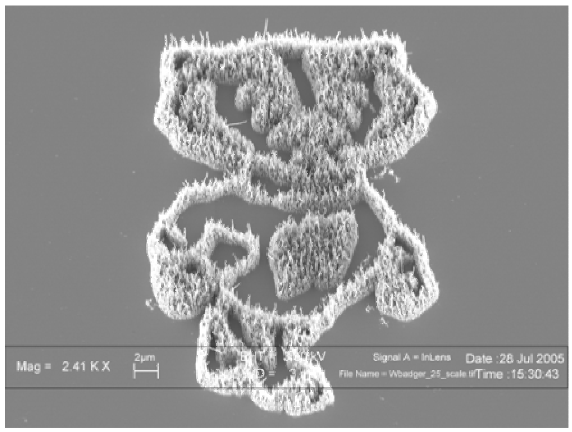


Image J
http://rsb.info.nih.gov/ij/applets.html

ImageJ with JAVA
http://rsb.info.nih.gov/ij/ImageJ.jnlp

Biofluorescence:GFP
http://www.loci.wisc.edu/optical/probes.html
http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP-1.htm

Teaching GFP:
http://www.wisc.edu/wistep/teach/pdf/explore_gfp/text.pdf
http://www.wisc.edu/wistep/teach/pdf/explore_gfp/append.pdf

Alba Activity
https://mywebspace.wisc.edu/djkelley/web/Alba.doc

Alba
http://www.ekac.org/gfpbunny.html#gfpbunnyanchor

Eduardo Kac and Alba, the fluorescent bunny.	Alba, the GFP Bunny

Photo: Chrystelle Fontaine

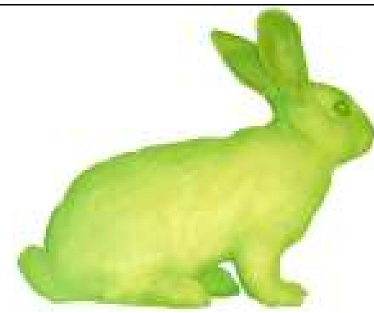
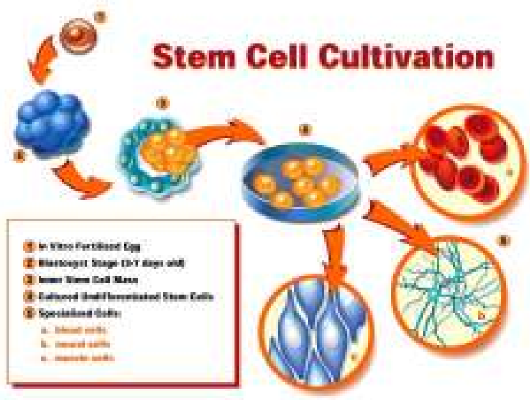


Photo: Chrystelle Fontaine

Confocal imaging
http://loci.wisc.edu/confocal/confocal.html

Fluorescence Imaging
http://www.microscopyu.com/articles/fluorescence/index.html

Wisconsin Embryonic Stem Cell Diagram:
http://www.news.wisc.edu/packages/stemcells/illustration.html



Discussion Paper:
Zwaka TP, Thomson JA.
Differentiation of human embryonic stem cells occurs through symmetric cell division.Stem Cells. 2005 Feb;23(2):146-9.
http://stemcells.alphamedpress.org/cgi/content/full/23/2/146

E. Explore Websites

Brainmaps
http://brainmaps.org/

Visible Human Project
http://www.nlm.nih.gov/research/visible/visible_human.html

Anatquest Viewer
http://anatline.nlm.nih.gov/index.html

National Museum of Health and Medicine Brain Collections
-Video Overview:
http://nmhm.washingtondc.museum/collections/neuro/NMHM-PBS.mpg
-UW-Madison Wally Welker Comparative Anatomy Collection,

http://brainmuseum.org/
-The Navigable Atlas of the Human Brain using the Yakovlev-Haleem Collection
http://www.msu.edu/~brains/humanatlas/

NIH Videocast
Demystifying Medicine - Imaging: A New Frontier for Organs and Cells
Elias Zerhouni (OD) and Jennifer Lippincott-Schwartz (NICHD)
Electronic Links: http://videocast.nih.gov/launch.asp?13093

The Human Brain: Cerebrum, Lobes and Cortical Regions
by Ethan Blanchette (Anatomy and advanced biology, Harvard)
http://outreach.mcb.harvard.edu/teachers/Summer05/EthanBlanchette/Human_brain.ppt

X ray, MRI, CT, PET
http://www.mos.org/doc/1921

Bone Density
http://science.exeter.edu/jekstrom/LABS/LABS.html

Phineas Gage
http://www.neurosurgery.org/cybermuseum/pre20th/crowbar/crowbar.html
http://content.nejm.org/cgi/content/full/351/23/e21/DC1

PET
Handouts: http://science.education.nih.gov/supplements/nih2/addiction/guide/pdfs/master1.1-1.7.pdf
Video: http://science.education.nih.gov/supplements/nih2/addiction/activities/lesson1_pet.htm

Contrast agents: NIH MICAD Database
http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/micad/home.html

Understanding Dimensions in Biology
http://www.loci.wisc.edu/cambio/bio.html

F. Background Reading

Massoud TF, Gambhir SS.
Molecular imaging in living subjects: seeing fundamental biological processes in a new light.
Genes Dev. 2003 Mar 1;17(5):545-80. Review. No abstract available.
PMID: 12629038
http://www.genesdev.org/cgi/content/full/17/5/545

Cassidy PJ, Radda GK.
Molecular imaging perspectives.
J R Soc Interface. 2005 Jun 22;2(3):133-44. Review.
PMID: 16849174
http://www.journals.royalsoc.ac.uk/media/52lwqlyrwmndhul3hjf3/contributions/g/h/y/g/ghyg583q94pa9bay_html/fulltext.html

From Bones to Atoms: Imaging Nature across Dimensions
http://www.mih.unibas.ch/Booklet/Booklet96/Booklet96.html

Science Web Extra: Biological Imaging 4 April 2003 Vol 300,
Issue 5616, Pages 1-196
http://www.sciencemag.org/feature/data/bioimaging/index.dtl

Nature Cell Biology Web Focus: Imaging in Cell Biology
http://www.nature.com/focus/cellbioimaging/index.html

G. Additional Resources

Wisconsin Histology Images
http://histology.med.wisc.edu/histo/uw/htm/ttoc.htm

Wisconsin Gross Anatomy Images
http://www.anatomy.wisc.edu/courses/gross/

Wisconsin Radiology Tutor
http://www.radiology.wisc.edu/education/forStudents/neuroradiology/NeuroRad/TOC.htm

Wisconsin Xenopus Neurulation Video
http://worms.zoology.wisc.edu/frogs/neuru/neuru_xen_timel.html

Wisconsin Electron Micrograph Library: DNA, DNA-Protein complexes & Virus
http://www.biochem.wisc.edu/inman/empics/

Wisconsin Stem Cell Images
http://www.news.wisc.edu/packages/stemcells/labphotos.html

Wisconsin Laboratory for Optical and Computational Instrumentation (LOCI)
http://www.loci.wisc.edu

Wisconsin W.M. Keck Laboratory for Biological Imaging
http://www.keck.bioimaging.wisc.edu/

Wisconsin Virus World
http://rhino.bocklabs.wisc.edu/virusworld

Wisconsin Waisman Laboratory for Brain Imaging and Behavior
http://brainimaging.waisman.wisc.edu/

Wisconsin Virtual Foliage Homepage
http://botit.botany.wisc.edu/

Wisconsin Microscopy
http://www.microscopy.wisc.edu/

Wisconsin Biological & Biomaterials Preparation, Imaging, and Characterization Facility
http://www.ansci.wisc.edu/facstaff/Faculty/pages/albrecht/albrecht_web/Programs/microscopy/home.html

Wisconsin Microbial World (Ken Todar)
http://www.bact.wisc.edu/themicrobialworld/homepage.html

Society for Neuroscience Database for Images and Atlases
http://ndg.sfn.org/

Virtual Microscope
http://virtual.itg.uiuc.edu/

NASA Remote Sensing Tutorial: Medical Imaging
http://rst.gsfc.nasa.gov/Intro/Part2_26b.html

National Institute of Biomedical Imaging and Bioengineering (NBIB)
http://www.nibib1.nih.gov/HealthEdu/ScienceEdu/Resources/Parents

NBIB Picture and Video Gallery
http://www.nibib.nih.gov/publicPage.cfm?section=gallery&action=view

Image & Video Library of The American Society for Cell Biology (ASCB)
http://cellimages.ascb.org/

Science Museum: Imaging the Living Brain
http://www.sciencemuseum.org.uk/exhibitions/brain/178.asp

Microbiology Video Library
http://www-micro.msb.le.ac.uk/video/

VII. STUDENT MATERIALS

A. Day 1-Nanoimaging

NanoBucky

Learning goals:
1) Understand nanoscale imaging.
2) Understand how imaging provides scientists with ways of knowing biological processes.
3) Recognize that imaging is a quantitative tool in biology by observing, measuring, and interpreting biological images

Students meet the learning goals when they understand the relationship between biological scale and imaging scale, when they can answer questions about the way images provide ways of knowing biology, and when they can quantify images of Nanobucky with NIH Image J.

Instructions:
In small groups of 4 to 6, students will complete the following activity. Use the questions to guide you through this activity.

Duration:
This activity should take 30 minutes.

Credits:
This activity was developed from online resources:

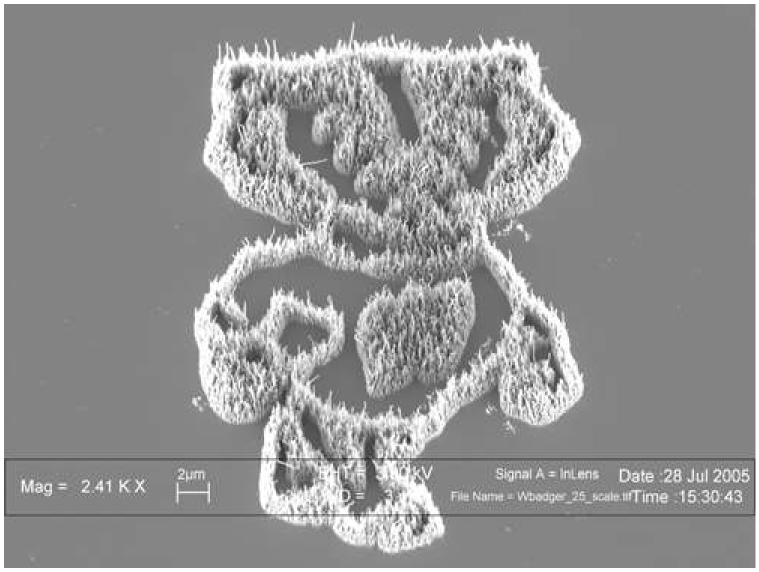
1) Patterned Nanofibers: The making of "NanoBucky"
Sarah Baker, Kiu-Yuen Tse, Jeremy Streifer, Matthew Marcus, and Prof. Robert Hamers
Hamers Research Group,UW-Madison
http://hamers.chem.wisc.edu/research/nanofibers/index2.htm

2) NIH Image J
http://rsb.info.nih.gov/ij

3) Image J Documentation Wiki
http://imagejdocu.tudor.lu/imagej-documentation-wiki

Nanobucky is a fun example of the ability to control the synthesis of nanoscale materials such as carbon nanofibers. Nanobucky is made entirely from tiny "hairs" of carbon nanofibers.

Nanobucky



The carbon nanofibers that make up Bucky are of great interest for practical applications such as chemical and biological sensing and as high surface-area materials for use in applications such as energy storage. So, while NanoBucky is fun, there is some serious science behind making structures such as this.

Students' goal is to quantify the height and image intensity of Nanobucky using NIH Image J Software.

Student Directions:
Start Image J directly or with the JAVA servlet http://rsb.info.nih.gov/ij/ImageJ.jnlp

Load the image File>Open>nanobucky1.gif

Set the scale of your measurements

Click the straight line tool on the toolbar and place a line over the image scale bar.

Image> Zoom In may be helpful

Click **Analyze>Set Scale**

Use this **Set Scale** dialog to define the spatial scale of the active image so measurement results can be presented in calibrated units, such as millimeters.

Enter the known distance and unit of measurement, then click OK. ImageJ will have automatically filled in the Distance in Pixels field based on the length of the line selection.

Set **Distance in Pixels** to zero to revert to pixel measurements.

Setting **Pixel Aspect Ratio** to a value other than 1.0 enables support for different horizontal and vertical spatial scales, for example 100 pixels/cm horizontally and 95 pixels/cm vertically. . In this exercise leave a number 1 in the dialog box.

When **Global** is checked, the scale defined in this dialog is used for all images instead of just the active image. Check the global box

Next Set Measurements by clicking **Analyze> Set Measurements**

Use this dialog box to specify which measurements are recorded by Analyze/Measure in the next step

Make sure the following options are selected.

Area - Area of selection in square pixels. Area is in calibrated units, such as square millimeters, if Analyze>Set Scale was used to spatially calibrate the image.

Mean Gray Value - Average gray value within the selection. This is the sum of the gray values of all the pixels in the selection divided by the number of pixels. Reported in calibrated units (e.g., optical density) if Analyze>Calibrate was used to calibrate the image. For RGB images, the mean is calculated by converting each pixel to grayscale using the formula gray=0.299*red+0.587*green+0.114*blue if "Weighted RGB Conversion" is checked in Edit>Options>Conversions or the formula gray=(red+green+blue)/3 if not checked.

Min & Max Gray Value - Minimum and maximum gray values within the selection.

Feret's Diameter - The longest distance between any two points along the selection boundary. Also known as the caliper length.

Integrated Density - The sum of the values of the pixels in the image or selection. This is equivalent to the product of Area and Mean Gray Value.

Decimal Places - This is the number of digits to the right of the decimal point in real numbers displayed in the results table and in histogram windows. Set this to 3.

Draw a Region of Interest Measurement

Draw a vertical line from NanoBucky's head to toe. Try to select the maximum distance you can find.

Analyze your Measurement

Click Analyze>Measure

Based on the selection type, the Measure command calculates and displays either area statistics, line lengths and angles, or point coordinates.

Area statistics are calculated if there is no selection or if a subregion of the image has been selected using one of the first four (area selection) tools in the tool bar. Calculates line length and angle if a line selection has been created using one of the three line selection tools.

With line selections, the following parameters can be recorded: length, angle (straight lines only), mean, standard deviation, mode, min, max and bounding rectangle (v1.34l or later). The mean, standard deviation, etc. are calculated from the values of the pixels along the line.

1) Record the line results here:

Students: Repeat the measurement and analysis process but now with an Ellipse that just encircles NanoBucky

2) Record ellipse results here:

3) How tall is NanoBucky?

4) What is the mean grey value based on your ellipse measurements?

Day 2 - System And Cellular Molecular Imaging Using EGFP

1. Alba: The EGFP Bunny

Learning objectives:
1) Understand macroscale molecular imaging techniques.
2) Understand how imaging provides scientists with ways of knowing biological processes.
3) Recognize that imaging is a quantitative tool in biology by observing, measuring, and interpreting biological images

Instructions:
In small groups of 4 to 6, complete the following activity. Use the questions to guide you through this activity.
Duration:
This activity should take 10 –15 minutes.

Credits:
This activity was developed from online resources:

1) USING THE GREEN FLOURESCENT PROTEIN TO TEACH MOLECULAR LITERACY: THE FLOW OF GENETIC INFORMATION AT THE MOLECULAR LEVEL; Michael H. Patrick, Ph.D and Tim Herman, Ph.D.; Wisconsin Teacher Enhancement Program, UW-Madison; Center for BioMolecular Modeling, Milwaukee School of Engineering
http://www.wisc.edu/wistep/teach/pdf/explore_gfp/text.pdf

2) GFP Bunny (2000), text by Eduardo Kac.
http://www.ekac.org/gfpbunny.html#gfpbunnyanchor

Green fluorescent protein was identified in 1971 as the protein responsible for the green fluorescence of the Pacific Northwest jellyfish, Aequoria Victoria. The protein was purified and the structure determined in 1996. Around the same time, the gene encoding this protein was cloned and introduced into a variety of other cells, from bacteria to human cells." "Alba", a fluorescent bunny, is an albino (white) rabbit with no skin pigment. Alba was engineered using EGFP, an enhanced GFP that provides greater intensity fluorescence than GFP. Under different lighting conditions, Alba appears to change colors.

ALBA

Photo: Chrystelle Fontaine

Use the information below to answer the following questions:

Based on the photos above what color is Alba?

Based on the emission spectra for EGFP below, what color light does EGFP emit?

Based on the excitation spectra for EGFP below, what wavelength of light can excite EGFP?

What color light must shine on Alba in order for Alba to glow? What color should Alba glow?

Does Alba glow in the dark? If not, why not? When will Alba glow? Does Alba glow all the time?

	Residue changes	Extinction coefficient ($M^{-1}\ cm^{-1}$)	Quantum yield (%)	Excitation peak (nm)	Emission peak (nm)
EBFP	F64L, Y66H, Y145F	31,000	25	383	445
ECFP	S65A, Y66W, S72A, N1461I, M153T, V163A	26,000	40	434	477
EGFP	F64L, S65T	55,000	60	489	508
EYFP	S65G, V68L, S72A, T203Y	84,000	61	514	527
dsRed		72,500	68	558	583

http://jcs.biologists.org/cgi/content/full/114/5/837
Journal of Cell Science 114, 837-838 (2001)

Color	Wavelength (nm)	Frequency (THz)	1 terahertz (THz) $= 10^3$ GHz $= 10^6$ MHz $= 10^{12}$ Hz
Red	780 - 622	384 - 482	
Orange	622 - 597	482 - 503	
Yellow	597 - 577	503 - 520	1 nm
Green	577 - 492	520 - 610	$= 10^{-3}$ um
Blue	492 - 455	610 - 659	$= 10^{-6}$ mm
Violet	455 - 390	659 - 769	$= 10^{-9}$ m
The white light is a mixture of the colors of the visible spectra.			

http://www.usbyte.com/common/approximate_wavelength.htm

ALBA

Photo: Chrystelle Fontaine

B. Day 2 - System And Cellular Molecular Imaging Using EGFP

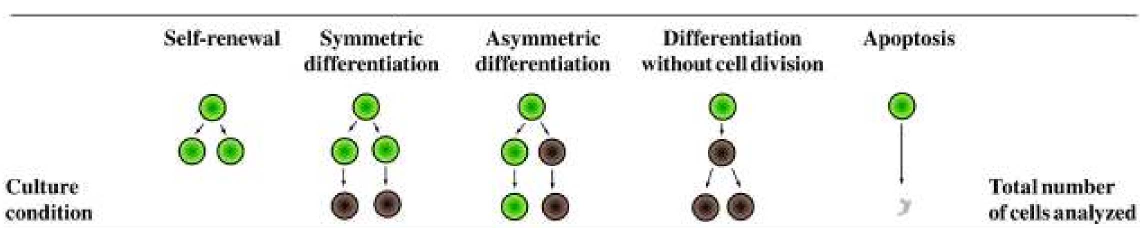
2. EGFP in Embryonic Stem Cells

Learning objectives:
1) Understand microscale molecular imaging techniques.
2) Understand how imaging provides scientists with ways of knowing biological processes.
3) Recognize that imaging is a quantitative tool in biology by observing, measuring, and interpreting biological images
4) Demonstrate proficiency with the analyze tool in NIH Image J software by quantifying biological images.
5) Understand the language of biological imaging.

Instructions:
In small groups of 4 to 6, complete the following activity. Use the questions to guide you through this activity.
Duration:
This activity should take 10 –15 minutes.
Credits:
This activity was developed from online resources:

1) Zwaka TP, Thomson JA.
Differentiation of human embryonic stem cells occurs through symmetric cell division.
Stem Cells. 2005 Feb;23(2):146-9.
PMID: 15671139
http://stemcells.alphamedpress.org/cgi/content/full/23/2/146

Pluripotent ES cells can divide and differentiate into any cell type. Oct4 is a protein solely expressed in pluripotent cells. By tagging the protein with EGFP, this protein becomes a marker for pluripotency and can help determine the mechanism by which pluripotent cells divide and differentiate. Retinoic acid (RA) induces pluripotent cells to differentiate.



"We tracked the differentiation state of human embryonic stem cells using an Oct4-eGFP knock-in cell line. Oct4 is a central regulator of pluripotency. It is expressed exclusively in the pluripotent cells of the embryo. We used time-lapse videomicroscopy over 5 days to track phase-contrast images. EGFP expression levels…are an indicator of Oct4 expression. Figure 1A depicts an undifferentiated, Oct4+, human embryonic stem cell (arrow) undergoing a cell division. Both daughter cells show synchronous down regulation of eGFP, and therefore Oct4. Differentiation was also indicated by the change in morphology observed in the corresponding phase-contrast images. In total, we tracked… 60 individual cells for 5 days under each of the four conditions (Fig. 1B). To determine eGFP fluorescence, the shape of individual cells was determined and a region of interest (ROI) was defined. ROIs were transferred into the acquired fluorescence image…"

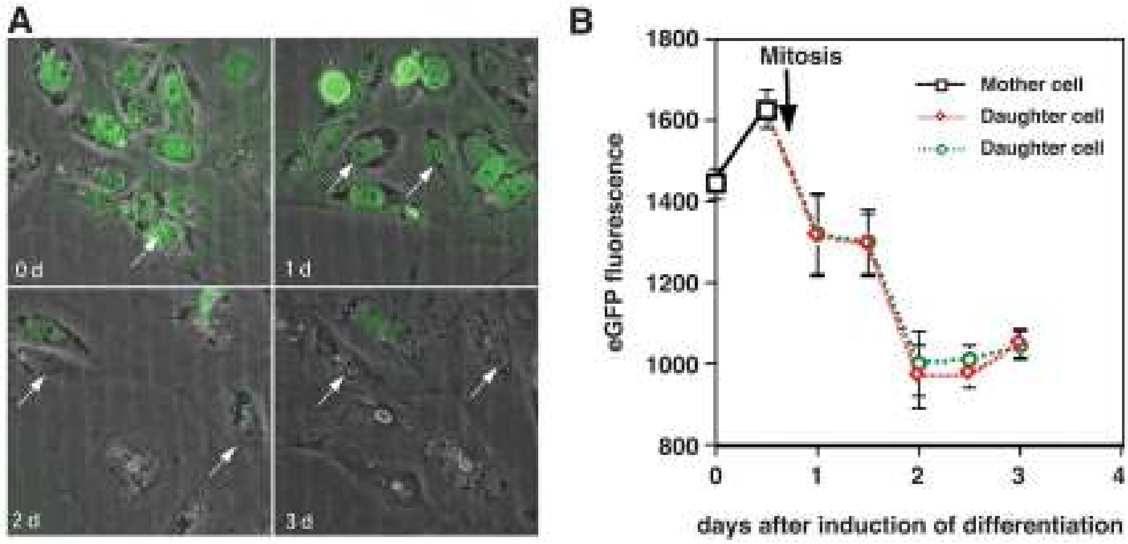


Figure 1. Human embryonic stem cells differentiate symmetrically. (A): Extracts from time-lapse analysis showing differentiation of one human embryonic stem cell colony after RA treatment. A representative cell and its two daughter cells are marked with arrows; green shows eGFP, reflecting the internal level of Oct4 in individual cells. Following cell division, both daughter cells down regulate eGFP and, therefore, Oct4 in the same way. (B): Mean eGFP fluorescence in one human embryonic stem cell after induction of differentiation with RA. The eGFP signal has been followed over time. The time point of cell division is marked. Three sequential images were used to determine eGFP fluorescence intensity for individual cells. Error bar, standard error of mean. Abbreviations: eGFP, enhanced green fluorescent protein; ES, embryonic stem; RA, retinoic acid.

What are the emission and excitation peaks for EGFP tagged Human Embryonic Stem Cells?

What is the EGFP being used to monitor?

Why was time lapse microscopy used?

How was the graph in panel B made and what does it show?

How do the scientists know that Oct4 expression is reduced in differentiated cells?

C. DAY 3 - Positron Emission Tomography (PET) Imaging Using 18-Fluorodeoxyglucose (^{18}FDG)

3D stereolithographic brain bodel and Phineas Gage skull model are available from David Nelson or the UW-Madison Biotechnology Center. This is to be used in conjunction with the medical imaging survey.

Learning goals:
1) Know that PET is an interdisciplinary imaging modality
2) Know that PET helps us understand the relationships between specific areas of the brain and what function they serve
3) Know that FDG PET measures metabolic activity
4) Know that PET uses radioactive compounds
5) Know that PET has clinical applications
6) Know imaging vocabulary: PET and FDG

The learning goals, which were addressed in PowerPoint presentations and online resources, need to be understood in order to answer the questions in this activity.

Instructions:
In small groups of 4 to 6, complete the following activity. Use the questions to guide you through this activity.

Duration:
This activity should take 10 –15 minutes.

Credits:
This activity was developed from online resources:

1) NIH Office of Science Education and the National Institute on Drug Abuse
NIH Curriculum Supplement Series on "The Brain: Understanding Neurobiology Through the Study of Addiction"
http://science.education.nih.gov/supplements/nih2/addiction/default.htm

2) UW-Madison Cyclotron/ Positron Emission Tomography Research Center
http://www.medsch.wisc.edu

Interpreting PET Images

This can be obtained by download from the NIH Office of Science Education and the National Institute on Drug Abuse NIH Curriculum Supplement Series on "The Brain: Understanding Neurobiology Through the Study of Addiction"
http://science.education.nih.gov/supplements/nih2/addiction/default.htm

The file containing the activity is located at:
http://science.education.nih.gov/supplements/nih2/addiction/guide/pdfs/master1.1-1.7.pdf

Clinical Case Application

Below are PET images collected at the University of Wisconsin-Madison Cyclotron/ Positron Emission Tomography Research Center (http://www.medsch.wisc.edu). Two patients were scanned using FDG while at rest. One of the patients is thought to have a tumor. On the images, white indicates greater glucose metabolism.

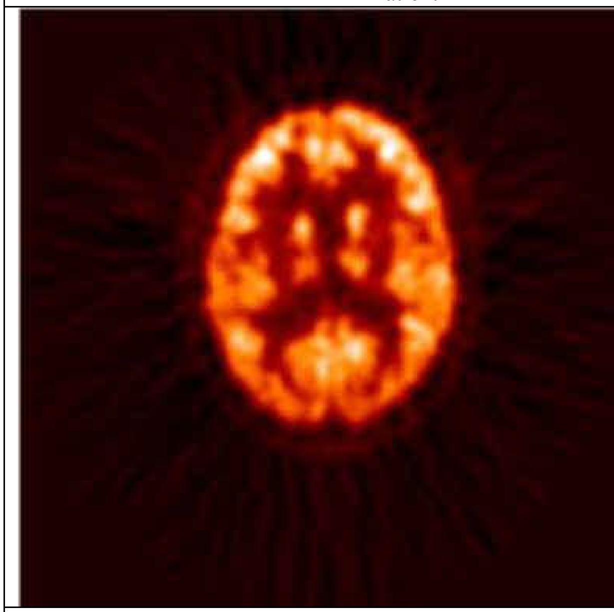
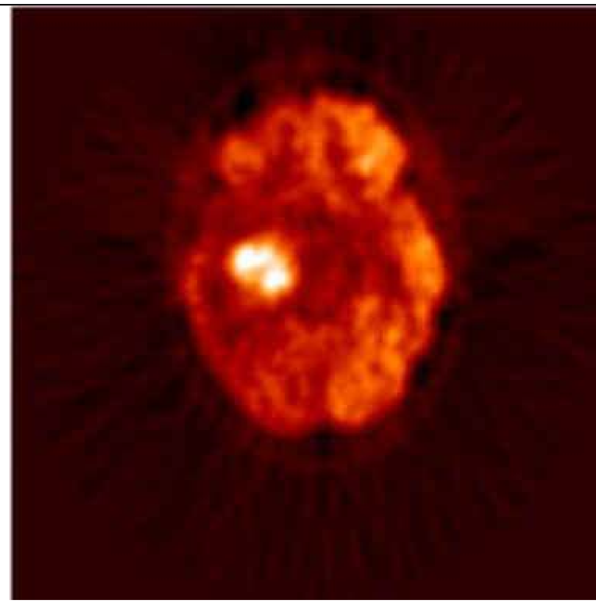
At approximately what level was each slice taken (a,b,c, or d)?

Patient 1:

Patient 2:

By comparing to the images in Set 1, circle which patient has a tumor?

Patient 1 OR Patient 2

Patient 1	Patient 2
	
http://www.medsch.wisc.edu/cycl/	