DRAFT White Paper on future directions in imaging

Executive summary

Imaging, extending from structural biology to functional imaging of organs and organisms, permeates all disciplines and specialties in biology. It also links biology to advances in physics and computer science and spans from physics to patients. There are areas of great strength at Harvard but also major lacunae. The whole is not always more than the sum of its parts. Cross-departmental and cross-institutional centers, rather than a new department or a single university committee, are the appropriate means to redress the weaknesses and reinforce the strengths. We propose that HMS affirm the mandate of the Center for Molecular and Cellular Dynamics (CMCD), create a similar organization in areas of imaging beyond the scope of CMCD, and make appointments (some jointly with SEAS and FAS) to fill current gaps. HMS must work out how to invest in activities at the hospitals as well as on the quad and must create incentives for improving communication among various outstanding imaging centers at MGH and in the Longwood area. Collaborations with MIT should be recognized and enhanced.

Imaging: definition and current state of the field

Imaging and genetics are the methodological correlates of the traditional structure and function poles of biology. We therefore define "imaging" very broadly, extending from structural biology (imaging of molecules, molecular complexes, and subcellular assemblies) to functional imaging of organs and organisms (e.g., fMRI). The territory includes rapdily advancing fields such as high-resolution, live-cell imaging, multi-photon imaging of living tissues, new extensions of magnetic resonance imaging, and so forth. This white paper will inevitably give an inadequate account of many important areas.

The report of the Tools and Technology Task Force includes recommendations about how best to enhance Harvard's strength in many areas of imaging. Those recommendations distinguish *(i) frontier research*; *(ii) development and appication of frontier discoveries*; *(iii) service core facilities* and their associated *training activities*. We focus here on the frontiers in imaging and especially on areas in which applications to biology and medicine are just becoming possible. Stength at the frontier is, in the long run, essential for collaborative application and for cutting-edge core facilities.

Frontiers in imaging extend from physics to patients. Important areas for biological and medical applications of imaging include the following.

Molecules and macromolecular assemblies. Single-molecule approaches are rapidly changing the way we think about structure-function studies of proteins and protein assemblies. Superresolution methods in light microscopy and cryoelectron tomography will close the resolution gap between molecular electron microscopy and the current state-of-the-art in live-cell imaging (see next paragraph) and complete the spectrum of multi-resolution ("hybrid") approaches needed to relate atomic-resolution information to events in living cells. Applications of more traditional structural methods (x-ray crystallography and molecular EM) to membrane proteins (many of which are importnat drug targets) have also come into their own, from advances in recombinant expression and biochemistry of membrane proteins and in technologies for working with very small quantities of protein. Other areas ripe for application include structure-based vaccine design and mechanistic enzymology at the single-molecule level (leading ultimately to advanced structure- and mechanism-baed pharmacology).

Subcellular structure and organization. High-resolution definition of supramolecular events in time and space in living cells has become a realistic goal, because of on-going advances in optical hardware and especially in image-analysis software, as well as the creation of genetically encoded and physiologically compatible probes (fluorophores). It has become possible to study intracellular dynamics and cellular remodeling across timescales ranging from 1-10 msec to 1 hr or longer and at spatial resolutions (ultimately in three dimensions) comparable to the diameters of individual virus

particles or of synaptic vesicles (50 -100 nm). Exciting applications are to: cellular dynamics (from molecular motors and membrane traffic to cell division and cell-cell interactions), cell-pathogen interactions, formation of intercellular synapses in the immune system, subcellular organization of neurons in development and synapse formation, etc. The field is limited by the range of available fluorescent probes, offering major opportunities for collaboration between chemical biologists and cell biologists. The goal of true "molecular movies" of complex intracellular processes, such as vesicular transport in the Golgi or chromosome segregation, is realistic (on a 15-20 year time scale), and thus the goal of studying how drugs or pathogens alter these processes is also realistic.

Cells, tissues, and small model organisms. The study of developmental events in suitable model organisms will benefit greatly from on-going advances in non-linear light-microscopic (e.g., multi-photon) technology, as will the study of synapse formation as linked to learning and behavior. Coupling of imaging tools with physiological, metabolic, and behavioral measurements is clearly an area of great excitement in neuroscience. Channelrhodopsin is one example of a genetically encodable tool that will lead to new discovery. The intersection of neuroscience and imaging is the principal area to which the new HHMI Janelia Farm Campus is dedicated. In the clinical arena, diffuse optical tomography is already being applied to specific problems in breast cancer and brain imaging; advances in computational aspects of image analysis (to compensate even more powerfully for noise and scatter) and development of probes may substantially extend its range.

Materials and surfaces. Texture, charge density, hydrophobicity, and other properties of surfaces couple with receptor localization and motion to have a major influence on the interaction of cells and tissues with exogenous materials. AFM, STEM, two-photon, and other imaging modalities are relevant to characterizing these properties.

Small and large animals. MRI and PET are the principal tools. PET is a mature technology (except for its recent combination with MRI and the creation of corresponding double-labelled probes); MRI continues to advance as a technique. The most important frontiers, even in MRI, are probably at the level of probe development. The sequence mice --> rats --> non-human primates [--> humans] will probably lead to real translational advances, although the gap to humans is substantial because of the time required for development of clinically approved contrast agents. There are numerous problems in human pathobiology to study, if the gap between technology centers and clinicians with interesting and informative groups of patients can be bridged.

Common scientific challenges

Each of the research areas just outlined involves different applications and different ranges of imaging modalities, but innovations in one area will often aid another, as many of the problems of data handling and data analysis are similar. For any particular imaging method, we can distinguish *data acquisition*, *image storage*, *image reconstruction*, *image analysis* (e.g., segmentation), and *quantitative analysis* of sets or sequences of images. Data acquisition tends to be specific to the particular imaging method and biomedical application, but many of the other steps have common aspects. For example, each of the areas described above relies on increasingly massive data storage and retrieval and increasingly complex image-processing computations. Structural biology as an international community has a reasonably successful tradition of finding common solutions to these problems, but similar initiatives have been less successful in the other areas outlined above. Efforts such as the Open Microscopy Environment (www.openmicroscopy.org), for example, have had only limited traction so far, perhaps because most groups operate entirely with commercial software and have limited software sophistication. Likewise, in molecular imaging of animals and humans, images are not easily transferred from one platform to another. Harvard has an opportunity to lead in this domain.

Current strengths and weaknesses at Harvard

Strengths: In *frontier research* in imaging and imaging methods, HMS is particularly strong in structural biology and in key areas of non-invasive imaging of animals and humans. Innovation in these fields at Harvard has led to rapid collaborative dissemination and discovery – e.g., in the structural biology of membrane proteins and in applications of magnetic resonance imaging. HMS also has a number of outstanding groups applying state-of-the-art live-cell imaging technologies (developed elsewhere) to important problems in cell biology. Another Harvard strength that might be exploited is the primate center, to enhance imaging studies of non-human primates.

Organizational models for success: In structural biology, creation of the Armenise-Harvard Center for Structural Biology and its growth into the HMS Center for Molecular and Cellular Dynamics (CMCD) nucleated the assembly of a vigorous structural biology community, spawned an active single-molecule biophysics community (including groups in FAS, HMS, Brandeis and MIT), and generated a national facility for structural biology software support (SBGrid: www.SBGrid.org). CMCD, a center without walls and without a specifically defined "membership", is a good organizational model for fields in which the nucleus of a community is already present and in which there is identifiable leadership for maintaining and expanding that community. It covers the spectrum from frontier research to core facilities. Somewhat more conventionally organized centers at MGH have made that institution a powerhouse in animal and human imaging: the Wellman Center, the Center for Molecular Imaging, and the Athinoula A. Martinos Center – the last a collaboration with HST. These centers cover frontier research and collaborative applications in their

specialties; they are not intended as broadly accessible core facilities. (There is a newly organized, open-access Longwood small-animal imaging facility at BIDMC.) Each of these cases appears to have benefitted from substantial seed funding and focused leadership.

Weaknesses: Despite the excitement of new developments in live-cell fluorescence microscopy and in non-linear (multi-photon) microscopy of living tissues, and despite the very high level of activity in application of these approaches, as listed above, Harvard (all components, including SEAS and FAS) lacks both a major innovator in optical methods and/or image analysis and a leader in development of novel fluorescent probes. Moreover, a weakness across the university in computational biology (rectified in certain areas recently by creation of the HMS Systems' Biology Department) amplifies the absence of innovation in image analysis. A good argument can be made that the most important advances in imaging of living cells and tissues during the next decade will come from novel computational approaches (especially in image analysis but also including modes of data collection and instrumental control), coupled with more incremental advances in instrumentation (lasers, detectors, etc.). Thus, rectifying weaknesses in imaging will require attention to the overall issue of innovation in computational biology.

Limitations of current organizational models: (a) The relative isolation of the world-class imaging centers at MGH from other parts of HMS show that geographical and institutional fragmentation can be a severe problem; the success of CMCD shows that it need not be. (b) Without the presence of research at the frontier (level i) of a technologically driven field, it is difficult to maintain excellence at levels ii (development of applications and collaboration of technology experts with biologists or clinicians) and iii (service and training). Thus, the Nikon Imaging Center is an outstanding facility in the last category, but it has (necessarily) limited capacity to implement new methods, and it cannot provide more than turnkey analytical and software assistance, as the instruction and scientific leadership from above is missing.

Recommendations for HMS

Imaging permeates all disciplines and specialties. Cross-departmental, cross-faculty, and cross-institutional entities should therefore be encouraged. Some areas (structural biology; subcellular imaging; many areas of tissue imaging) should have many distributed locations. MRI and other modalities of small- and large-animal imaging might need so much investment that some degree of coordination and specialization would be appropriate. Software repositories and database sharing offer ways to link distributed laboratories. Links to SEAS are highly desirable.

From these observations, it follows that some further investment in existing entities (e.g., in departments such as BCMP, Cell Biology, and Neuroscience – or their future re-

embodiments) is needed, but that it will certainly not be sufficient. It also follows that a single new department or committee, either at HMS or in Allston, would also be inappropriate. *We therefore recommend that cross-departmental and cross-institutional centers be the principal mechanism for consolidating strengths and rectifying weaknesses in imaging*.

1. The spectrum from *structural biology to subcellular imaging* is already strong, with CMCD as an organizational focus. The mandate of CMCD in high-resolution optical microscopy should be enhanced, and the community strengthened by several critical appointments. (A list of fields would include the following areas: *new developments in optics*, probably jointly with SEAS; *computational image analysis*, e.g. in cell biology; *development of new fluorophores*, in collaboration with the chemical biology program; a further appointment in *contemporary electron microscopy*; *single-molecule biophysics*.)

2. Advanced imaging of cells, tissues and small model organisms would benefit from a cross-departmental and cross-institutional organization, similar to CMCD. Close links to CMCD would be valuable in areas of data management (e.g., a generalization of SBGrid). While neuroscience would be a major component of such an effort, its scope should not be restricted to that field. Immunology and developmental biology (outside of the nervous system) are obvious areas. As in subcellular imaging (with which some of the individuals may overlap), appointments to strengthen Harvard's effort in *computational methods of image analysis* and in *development of new fluorescent probe*s should be a priority.

3. Non-invasive imaging of animals and humans. There is an outstanding community at HMS, and the first key issue is how to make the whole greater than the sum of its parts. HMS must work out how to invest in research at the hospitals, in order to provide incentives for crosstalk among major groups and to stimulate critical appointments to benefit the broader community, not just the hospital in question. Strong links between MGH imaging centers and MIT are a strength, and HMS, the hospitals, and MIT should work out how to build even more creatively upon these interactions. A second issue, related to the first, is software and data sharing. Harvard has an opportunity to lead in this domain, if the right people can be brought together in a coherent way. Progress in this area could galvanize translational studies and help set national and international standards. This might be a useful initial mission of an umbrella organization, if the right leadership could be identified, as it involves scientific rather than institutional issues.

4. Cautionary notes: If additional cross-departmental and cross-institutional centers or programs are to be created, in any field, HMS must consider carefully how to structure them (without undermining the departments). If the centers are to be more than communities linked by intellectual ties, moral suasion, and pre-existing consensus, their leaders will need *direct access to the Dean* (rather than through their respective

department chairs) and *independent resources* (with which to fund infrastructure and provide supplementary startup funds for appointments in collaboration with a department). The Dean will need to retain central control of a reasonable amount of *space*, in order to accommodate infrastructure investments that do not fit easily into a single department. Finally, *reciprocity arrangements* with the affiliated hospitals will be essential for any of these efforts to succeed.

Appendix 1: Draft prepared by Stephen Harrison, based in part on work of the Tools and Technology imaging subgroup (Bernardo Sabatini, John Frangioni, David Corey, Bruce Rosen, Ralph Weissleder) and on direct consultation with Bernardo Sabatini, John Frangioni, Bruce Rosen, and Elazer Edelman. Modified in response to a discussion with members of the Biomedical Research Task Force on Feb. 27, 2008.

Appendix 2: Summary of Tools and Technology subgroup recommendations in Imaging.

(i) Appointments: recruit a leader in development of fundamental new optical imaging modalities; recruit new leaders in fluorophore/probe/reagent chemistry.

(ii) Create coherent communities (centers?) in imaging of cells, tissues, animals and humans, to parallel the coherence of the structural biology/single-molecule community.

(iii) Create more direct communication between leading PIs and the Dean, so that space and resources can be made available when needed to enhance cross-departmental efforts.

(iv) Improve communication through creation of an Office of Tools, Technology, and Facilities

(v) Strengthen existing core facilities for service and training (e.g., the Nikon imaging facility)