## BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
### DIRECT COSTS ONLY

<table>
<thead>
<tr>
<th>BUDGET CATEGORY TOTALS</th>
<th>INITIAL BUDGET PERIOD (from Form Page 4)</th>
<th>ADDITIONAL YEARS OF SUPPORT REQUESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>PERSONNEL: Salary and fringe benefits. Applicant organization only.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONSULTANT COSTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EQUIPMENT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUPPLIES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRAVEL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PATIENT CARE COSTS</td>
<td>INPATIENT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OUTPATIENT</td>
<td></td>
</tr>
<tr>
<td>ALTERATIONS AND RENOVATIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTHER EXPENSES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONSORTIUM/CONTRACTUAL COSTS</td>
<td>DIRECT</td>
<td></td>
</tr>
<tr>
<td>SUBTOTAL DIRECT COSTS</td>
<td>(Sum = Item 8a, Face Page)</td>
<td></td>
</tr>
<tr>
<td>CONSORTIUM/CONTRACTUAL COSTS</td>
<td>F&amp;A</td>
<td></td>
</tr>
<tr>
<td>TOTAL DIRECT COSTS</td>
<td>100,000,137,44</td>
<td>230,650</td>
</tr>
</tbody>
</table>

### JUSTIFICATION

OVERALL

Current year -28 direct costs support is $243,653. Excluding equipment, proposed direct costs for year -29 support are $280,809, an increase of approximately 15.25%, the excess over 13% due to routine salary growth during the 12-month, year -28 extension we needed to plan and develop the current proposal.

Long term personnel is currently supported and projected at a level that allows focussed progress without additional fund-raising. A new low-level but high-skills part-time position of computer/lab assistant is included. This assistant's support is largely compensated by Departmental coverage for 15% of Research Analyst's salary. We plan in the next year to submit an allied proposal emphasizing X-ray diffraction of muscle fibers as a guide to developing better technology for EM specimen...
Budget Justification (continued)
preservation including freeze-substitution and cryo-tomography. The two staff members (Research Associate and Research Analyst) have been with the lab 23-26 years. Their skill and initiative are critical for meeting the special demands of this project.

PERSONNEL (Applicant Organization):

Michael K. Reedy, Ph.D. (Principal Investigator - 50% effort/salary). PI support is proposed to continue current and planned PI effort. Our program is now entering the most promising and demanding phase of its entire 28+ year span. My commitment and expectations have never been higher; we are very well positioned for culminating achievements. As PI, I plan, schedule and review analysis of all experiments based on routine procedures, and participate myself in non-routine procedures like quick-freeze cryofixation, X-ray diffraction, muscle fiber mechanics, and preparing publications and grant proposals.

Mary C. Reedy, Ph.D. (Research Associate - 100% effort/salary). My closest collaborator since she joined my lab in 1977, is a superbly productive and talented investigator and electron microscopist, co-(often first) author on most of our publications, intensely on-task in manuscript preparation, pivotal in initiating and tending many of our lab's major collaborations, including several that bring her talents and our lab's special expertise to new areas where she is publishing independently of me. This is reflected in her publication list, which has flourished over the last 20 years as she wrote and led research under two MDA grants, while reaching out to seed new directions in muscle, cytoskeletal and motility research, in efforts to broaden our contribution and possible funding. This has nourished her CV substantially. Mary cuts all the ultrathin sections for our 3D EM-tomography work with K.A. Taylor, and collects all tilt-series EM data at both Duke and FSU. For EM thin-section analysis of our quick-frozen fibers Mary shares with me in performing final data collection and critical data review phases, including a major share of the data quality screening for which we use the PC video-capture and CRISP software () for quasioptical image diffraction and filtering. Her critical approach and exuberant experimental participation are vital to all our work, and central to all our EM effort on muscle, cryo and otherwise.

Carmen Lucaveche, B.S. (Research Analyst - 85% effort/salary). Ms. Lucaveche has managed my lab since 1974. Promoted from Sr. Research Technician to Research Analyst 11 years ago, she skillfully handles all stages of specimen preparation from dissection to freeze-substitution, thin sectioning, and electron microscopy and does all special darkroom work. She is now co-author on a majority of our projects, reflecting the initiative she takes in experimental design, conduct and analysis. Her careful record-keeping and exquisite thin-sectioning are irreplaceable, supported by detailed logs, maps and video-taped embedding records (made on the dissecting microscope) of each frozen fiber that she has made during our freeze-substitution, embedding and sectioning of the over 300 quick-frozen fibers produced with our collaborators in Philadelphia in recent years. Equally vital is her unfailing care and perseverance in getting technically optimum sections and EMs from all the frozen fibers, overcoming difficulties that required extra effort to compensate for patchy freezing. I have currently agreed to accept Departmental support for 15% of her effort to allow her to assume primary responsibility for EM training and services for outside users.

The first lab assistant position is filled by 2-4 undergraduate students, currently Messrs Ward and Colles, as part-time workers who relieve research staff of 20+ hr/wk routine darkroom work, dish-washing, animal care, Xeroxing and library errands. Cost varies, because qualified students are sometimes available in-term as work-study (80% salary paid by Duke), but about equally often good work-study students do not apply. Then, and always in summer term, and for special needs (e.g., programming the Mac in LabView, ) we must pay 100% of $7-13/hr.

The second lab assistant position for 15 hr/wk will continue Mr. Rodrigo Cordova, available in
Budget Justification (continued)

PC and Mac computer problems and procedures. He has been crucially helpful over the past 2 years in keeping us on-line and well-advised on new choices, upgrades and trends.

KEY PERSONNEL (Outside Applicant Organization)

Progress under the 5 AIMS of this proposal is more than 50% dependent on experiments that could not be contemplated without the expertise and collaboration of the eight non-Duke Key Personnel listed below.

· Kenneth A. Taylor has been our closest and most valued collaborator since he came to Duke in 1981, and this continues since his 1995 move to FSU (Tallahassee). His software and computers transform our EM data from thin sections into 3-D tomographic reconstructions into which we can dock molecular models. The $2M electron microscopes that FSU helped Ken acquire after Duke declined to are now fitted with 2k² CCDs that speed up tomography 100X over previous film-based methods, drawing us more and more to make regular visits to his lab to perform the lion's share of data collection, in addition to our sessions for joint interpretation, model fitting and manuscript preparation. Ken's contribution is crucial for the 3-D developments in all but AIM 5. Effort is on an "as needed" basis without salary.

· Yale Goldman and Clara Franzini-Armstrong at U. Penn, who supervised and published in the 1990s on the definitive EM analysis of quick-frozen contracting frog muscle, together with expert instrument-maker Joe Pili, developed and maintain the specialized slam-freezing equipment that records tension and controls length of single fibers up to the instant of freezing. This instrument, its proposed evolution to accommodate plunge-freezing, their assistance and good will during 2 week muscle freezing-runs 1-2x yearly, and their active role in manuscript writing give irreplaceable support for AIMS 1-5. Effort for both is on an "as needed" basis without salary.

· Tom Irving works closely with us at the low-angle BioCAT beamline at APS/Argonne, bringing 20 years' experience in muscle diffraction to bear on our collaboration and on continued muscle-helpful refinements of already stunning beam quality and detector performance (AIMS 1-5). Effort is on an "as needed" basis without salary.

· P. Bryant Chase at the UW in Seattle will bring his authority and expertise on effects of phosphate transition-state analogs like vanadate or AlF₄⁻ (ADP裴 analogs) in vertebrate muscle to bear on defining the peculiarities of IFM responses to these important agents (AIM 2). Effort is on an "as needed" basis without salary.

· Richard Tregear supports us in IFM mechanics, X-ray modeling, and fiber diffraction with a lifetime's experience and a teenager's vigor, expertly managing insect fiber mechanics during our 2-week muscle quick-freeze runs and we hope in our future synchrotron X-ray runs as well (AIMs 1,2). Effort is on an "as needed" basis without salary.

· John Squire's lab at Imperial College, London, has developed the only computational approach currently available which has been shown capable of objectively and automatically searching out IFM thick filament and A-band structural models that will uniquely account for muscle X-ray patterns (AIM 5). Effort is on an "as needed" basis without salary.

· Vincenzo Lombardi's lab in Florence houses the only workers in the world who have the requisite instrumentation and experience to perform the sub-millisecond time-resolved combination of single muscle fiber mechanics, length changes and stroboscopic X-ray diffraction needed as an independent structural control on our EM of millisecond-timed parallel experiments quick-frozen in the Goldman lab at U. Penn (AIMs 1,2). Effort is on an "as needed" basis without salary.

EQUIPMENT ($51,873):

Three equipment purchases are budgeted. Since they take the first year budget well past 110% of the previous year's budget, justifying detail is provided below. Each enables one of the three critical...
EQUIPMENT ITEM 1: ($13,113)

We will add **physiology equipment** for muscle fibers to expand our testing, temperature control and **X-ray diffraction capabilities**. This covers two sets of add-on modules from Dr. G•th, Heidelberg to integrate with the G•th Muscle Research Workstation (®) that we have used for 10 years now. This system is used to screen skinned & glycerinated muscle fibers for quality and to measure mechanical responses in standard and novel physiological states. **The first G•th add-on module will enable simpler and higher throughput** in our Duke lab. It consists of a multi-cup temperature-controlled solution changer that allows convenient fiber mounting and rapid positive change of solution between any of 10 different stirred Teflon cups holding 0.5-1.0 ml. This design inherently overcomes problems and time-sinks which have for 10 years discouraged all but the PI and one research assistant from learning to operate the flow-through perfusion cuvette now in use; even expert users must battle the near-continuous surface-tension artifacts and maddening delays and timing uncertainty during solution changes. A major goal of the new design will be to enable essential fiber testing and solution testing by Research Analyst Lucaveche and others, so that these tests are not all on the PI's dance card during urgent preparations for experiments away at Argonne, U. Penn and Florence. This module set **includes a faster new force transducer** with high (1.6 KHz) resonance frequency, enabling tension change measurements within ~0.5 ms after a length step. When integrated with upgrades we bought in 1999 to shorten time responses in the force-transducer amplifier and length control feedback regulator, this faster transducer will finally let us enter the critical Lombardi-Piazzesi-Irving timing regime (= Huxley-Simmons regime) of submillisecond fiber mechanics that remains just hidden by the artifacts of our present 2-3 ms response time. This in turn will enable us at Duke to develop and extend collaborative insights and protocols emerging from our visits to Lombardi's lab in Florence. Otherwise, the design and control of time-resolved structural experiments on insect flight muscle remains much too dependent on my limited access to their custom-built system with its unexcelled 0.05 msec response time.

**The second G•th add-on module set is an X-ray diffraction cuvette** for mechanical monitoring and perfusion of muscle fibers during X-ray diffraction, **coupled with a Peltier device temperature control**. The prototype G•th room temperature X-ray cell we used at Daresbury [Tregear, 1998 #12653] was a constant struggle with leaks and unstable windows. Its greatly improved present incarnation, kindly loaned by Barry Millman (U. Guelph, Ontario) for my Aug. 1999 beamtime at APS/Argonne, was a joy to use, and allowed temperatures from 2¡C to 37¡C. Millman's system is now in use by students at Guelph so not available. Moreover, I need a special-order version (G•th has agreed to fill the order) that combines a different built-in Peltier unit with a special controller module to reliably reach the -5¡ to +50¡ range my experiments will require. Supported by a Lauda tempering circulator for the Peltier heat exchanger, this system will bring the same range of temperature control to my benchtop and light microscope cuvettes, so that we can readily develop at Duke the protocols and background for Argonne/APS X-ray and U. Penn. freeze-trapping of structural states stabilized at ~0¡ and ~40¡C.

EQUIPMENT ITEM 2 ($24,760):

Funds requested for a **Leica EM CPC plunge-freeze workstation** (plunge-freeze workstation, cryostat control panel, LN₂ Dewar with autofill system; see ). Compact and easily transported by car, this cryo-workstation will standardize freeze-plunge velocity and timing and will stably cryostat the immediate specimen environment at any desired level between 77-300¡K. It will be adapted to incorporate plunge-freeze capability into the slam-freezer used at U. Penn for EM cryofixation of muscle fibers. It will also be taken to Argonne National Labs for on-site plunge-freezing of muscle fibers for cryo-X-ray-diffraction at the APS synchrotron source. We feel it crucial to explore the plunge-freezing alternative in parallel with continued slam-freezing, to help evaluate and avoid impact-shock artifacts peculiar to the impact-freezing which so far has provided our main method of muscle cryofixation. We expect synchrotron X-ray diffraction of flash-frozen muscle fibers to revolutionize collection and quality of
Budget Justification (continued)
the impact-freezing attachment, reducing the price from $35,000 to $25,000. Our experience over the past year at Duke with the smooth, stable wide-range cryostatting of the Leica AFM (see ) for freeze substitution has convinced us that the near-identical cryostat design of the Leica CPC requested here will be very satisfactory.

EQUIPMENT ITEM 3 ($14,000):
From discussion with machinist-instrument makers at Duke and Penn, we estimate that $14,000 will fund the following improvements in the drop-freezer (DF) in the Goldman & Franzini-Armstrong labs at U. Penn. These will all be ordered from and fabricated in the instrument-makers machine shop at U. Penn.

DF Item 1): for pre-freeze temperature & humidity control: & LN2 tanks, micro-dissecting microscope, and 3-4 smallish colleagues into (or very near to) walk-in cold rooms or warm rooms for a couple of days. Bringing temperature and humidity control to the drop-freezer, rather than vice versa, offers a much more versatile and durable option.

DF Item 2): for micro-plumbing (J-shaped tubes of 22-26 gauge stainless steel tubing) contrived to manage solution changes and suctioning on the mounted, inverted freezing head. This will perform 1-2 full cycles of sol’n change, followed by the obligatory suctioning of excess buffer from fiber and agar gel pad to eliminate aqueous surface film that would delay ultra-rapid freezing at the muscle fiber surface. The micro-plumbing manifold must be spring-retracted to clear the drop path 1-2 seconds before the drop begins. Initially, fluid delivery, vacuum-switched suctioning, and manifold withdrawal will be manually performed while observing the fiber and the inverted freezing head from below with a dissecting microscope. But rather soon, we will want hardware and software to automate these procedures under computer-timed control.

DF Item 3): for adapting the fiber support head and micro-plumbing systems for plunge-freezing [glucose cryo-protected] fibers into liquid cryogen, traditionally 20-30 ml of liquefied ethane-propane held at -190¡C. This device will be loaned for weeks/months at a time to the U. Penn. labs of Goldman & Franzini-Armstrong to enable their machine shop to adapt it to sit under the Heuser drop-freeze carrier rod as replacement for the slam-freezer metal mirror. The freezing head design must be partly re-engineered for the switch from slam- to plunge-freezing. The fiber will be suspended in air between vertical pins extending from force and length transducers. It will be physically supported by contact with a moist agar block until ~100-200 ms before freezing. Agar block and solution-exchange micro-plumbing will be retracted to clear the drop path at the last split second. The Leica EM CPC device requested above will cryostat the cryogen-filled plunge-pot at just above freezing (ethane/propane 3:1 mixtures stay liquid at LN2 boiling temperature).

SUPPLIES ($13,250):
$2750 for animals is primarily to ensure adequate live Lethocerus for flight muscle, from living bugs air-shipped from Jamaica, Florida and Thailand. Giant Lethocerus waterbugs are the mainstay for all our insect flight muscle work. Live bugs eat small goldfish and can survive 3-9 months in the lab aquaria. Glycerinated flight muscle is good for 1-5 years, kept at -100¡C as we keep it. For quick-freeze cryofixation, I am uneasy with older stored muscles, and prefer the freshest possible material, so I try to get in two shipments annually. Breeding and rearing Lethocerus in the lab is not cost effective; it cost $300-500 per live adult bug over 2 -3 years in the one lab (White & Sparrow, York, UK) where it was forced to success.
Budget Justification (continued)
diffraction and image processing, and for general glassware.

$2,200 will cover cryostorage supplies, including $110/mo for liquid N used for cryofixation and specimen storage, $40/mo for dry ice for -80°C freeze, and $33/mo for Dewars large and small needing to replacement or repair.

$1500 is needed yearly for miscellaneous desktop Macintosh and PC software and upgrades.

$3300 for chemicals and EM supplies includes liquid cryogens, DI water, EM grids, fixatives, nucleotides and analogues and biochemical/physiology supplies.

TRAVEL $19,000

Travel for quick-freezing, 3D computer reconstruction, synchrotron X-ray work, X-ray modeling and high-resolution fiber mechanics has been remarkably enabling of broad progress over the last 4 years, and will become even moreso over the next 5 years.

$9,000 domestic travel includes $2500 to support attendance at annual Biophysical Society Meeting and triennial Gordon Conference for the PI plus Research Associate. The other $6500 is for research visits to collaborator’s labs by PI ± Research Associate as follows:

1-2x yearly to Philadelphia, total 3 weeks, for time-resolved slam-freezing of contracting muscle experiments in the collaboration with Goldman/ Franzini-Armstrong labs and for consulting and writing papers. Cost $800/week for PI including car rental.

3-4x yearly for up to 4 week total visits to Ken Taylor’s lab in Tallahassee for direct interactive work on 3D reconstructions, planning experiments, use of on-line CCD and computerized goniometer to collect EM tomographic tilt series, and writing papers. Cost $1200 week PI + RA incl. car rental.

· 1x yearly for 2-3 years to visit Bryant Chase in Seattle to document mechanical responses of IFM vs. rabbit skinned fibers to PO₄ analogues (ADPPi₆; where Pi = PO₄ analogs such as AlF₄⁻, vanadate, or BeFₓ).

1-2x yearly for 1-2 week total visits to the BioCAT undulator beamline at APS/Argonne, ( ); Cost $1200 /wk PI incl. car rental, hotel. Each of these is at least a 4-9 day trip costing 800-1200/week including car rental, meals for 1 or 2, and lodging (varies $0-200/wk). All serve collaborations with state-of-the art workers and labs, providing expertise and equipment which in each case would cost tens to hundreds of thousands of dollars to develop at Duke. None is more crucial than the freezing experiments at Penn, central to this proposal and proven by experience in the current Grant period to assure progress in very difficult experiments due to the highly advantageous fit of complementary expertise and facilities between our two labs. I dissect, help mount and freeze 35-75 fibers per 1-2 week visit, drive the frozen specimens back to Duke in LN₂ (precludes air travel) and supervise the 2-4 weeks' freeze-substitution at Duke. The work with Taylor in Florida is the major step in data reduction and interpretation, and our main road to objective analysis of the structures preserved and imaged in our EMs. Important new structural information is increasingly available from synchrotron X-ray diffraction using Argonne’s BioCAT low-angle beamline. This puts 10¹³ photons/s into a focal spot 40 x 250 μm, allowing 1-10 millisecond recording of the strongest dozen or so reflections from bundles of 3-12 fibers of glycerinated Lethocerus flight muscle. Bryant Chase’s experience will certify gold-standard Pi-analog mechanics in a field beset by subtleties.

The $10,000/yr foreign travel is on 3 counts a special necessity, 1) for bringing Richard Tregear to from UK to UPenn to expertly manage insect fiber mechanics during our 2-week muscle quick-freeze runs (he has no travel budget at all); 2) for X-ray diffraction of contracting insect flight muscle with Lombardi’s group (Florence), and 3) direct interaction with X-ray modeling in John Squire’s lab (London). Tregear’s unmatched experience with IFM is vital, and his lively energy is a mighty inspiration in our experiments. The time-resolved X-ray of IFM, crucial for correlation with our freezing work, is being.
Budget Justification (continued)

begun in 1997, and will be carried out at Grenoble, Daresbury or BioCAT/APS synchrotron sources depending on where we can get beamtime and optimum time-slice detector technology. Alternative travel funding via NATO travel grants is no longer available. Funds requested are to bring the Florence group and their remarkably travel-ruggedized equipment to APS/Argonne, or to go myself with a US collaborator and my physiological equipment to ESRF or Daresbury. Travel & cargo for 2 US or 3 Italian participants will be $3000-4000. Lodging and meals for 4-6 people for 8 days (3 days setup, 5 days beamtime) at the beamline will cost $100/day/person and total $3200-4800. Travel to support and participate in the computer X-ray modeling collaboration with John Squire’s London lab is estimated at $1750, for 1.5 weeks per year costs of travel, meals and lodging.

OTHER COSTS ($32,670):

$17,000 is necessary to pay the service contract on the Philips EM 420, our high-resolution, high-tilt, cryoEM instrument, no longer supported by other expert faculty, who have either left Duke or lack current funding. All occasional and non-expert users are restricted to the EM 301, supported by piecemeal Departmental and user-contributed funds. The EM 420 is our only Duke instrument with goniometer tilt capability, which is essential for our 3D work to supplement 3D data collection using Ken Taylor’s facilities at FSU (Tallahassee). We are the major and currently Duke’s only funded users of the EM 420. We have so far been unable to get partial EM support commitments from experienced users in neighboring Departments where their EM labs have closed down in recent years.

Other service contracts include $1750 for SGI computer and software maintenance, and $1,750 upkeep on fee-for-service for other items including vacuum evaporators, ultramicrotomes.

$2670 is for publication costs (including color 3D molecular graphics & stereograms) and journal subscriptions, Xeroxing, phone, E-mail, FAX and postage.

$2500 is needed to cover necessary repairs, upgrades and replacements as needed for aging -100¡ C cryorefrigerator (repairs cost $2300 in 1996) to store glycerinated insect muscles, and for desktop computers and physiological electronics.

$7000 for minor equipment includes diamond knives for ultramicrotomy ($3,000 each, 1 per year), computer upgrades and replacements at $3000/yr, and $1000/yr for computer peripherals and physiological electronics formerly budgeted as Equipment.

EXTENDED BUDGET PERIOD: (YEARS 2 - 5)

Personnel is projected at a 4% increase to keep up with Duke’s projected estimates. Supplies, travel, and other costs are also projected at a 4% increase annually to match expected cost rises in these categories.

EQUIPMENT: $5,000/yr is requested for equipment years 2-5.

Physiological and Computer equipment, to be identified as we require it to support research progress is projected here. (1) The first thing we are considering is the need for a $5000 (estimated shop costs) redesign & rebuild of our X-ray bench support cradle that holds the 2.5 kg muscle length-control motor with attached X-ray cuvette and force transducer. (1A) The first thing needed is a Thomsen rails system to allow easy precision-roller translation of the whole set-up (8-10 kg when bolted to its remote-control x-y-z translation stage) ~1.4 meters lateral to the beam path (without the heavy lifting now required!) so a sitting operator can efficiently change fiber bundles (a semi-micro job of removing the old, orienting and reinserting the new under stereo-microscopy observation) and then roll the set-up back to within ~0.2 mm of...
Budget Justification (continued)

bundle. (1B) This cradle also needs a half-circle mount to enable rapid and convenient rotation of fiber bundle orientation around the beam axis, by 90° from our preferred working position with meridian (i.e., fiber axis) horizontal, to the meridian-vertical orientation necessitated by some critically promising experiments. Beam focusing optics at Argonne/APS/BioCAT produce a focus that is 4-6X tighter vertically than horizontally. Therefore we must orient fibers for meridian vertical to sharpen interference splitting of the meridional 14.5 nm reflection, possibly the only X-ray signal of crossbridge tilting we may get from insect flight muscle. As another example, a $6000 pressurized 8-vessel perfusion manifold with hardware and software to enable remote control either manual or by computer () will tremendously simplify multiple solution change sequences (including T-jumps from reservoirs held at different temperatures) for the G•th muscle cuvettes, especially the X-ray cuvette. A borrowed minimal gravity-feed system made by this company decisively proved the concept on our Aug 1999 run at APS/Argonne. Solution changing presently requires closing beam shutters and opening the X-ray hutch to effect every change of solution using G•th's multi-chamber solution carousel. Reservoir size (5 ml wells) and the exceedingly stiff, heavy grease seal make remote control adaptation untenable for this solution changer. As a third example we are considering a 2400 dpi Xante 3G laser printer for $5500 to mate with our 2500 dpi AGFA scanner as we explore bypassing the wet photo-print darkroom for preparing hard-copy work-prints of 90-95% of our EM and X-ray images.