

DOE/ER/60522-6

**DEVELOPMENT AND APPLICATION OF PHOTSENSITIVE DEVICE
SYSTEMS TO STUDIES OF BIOLOGICAL AND ORGANIC MATERIALS**

Third Year Progress Report

DOE/ER/60522--6

For the Period January 1, 1992 - December 31, 1992

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MASTER

I) INTRODUCTION & SUMMARY

This report describes progress as of the third year of a 3-year DoE grant DE-FG-02-87ER60522 for the fiscal period 1/1/92 to 12/31/92. Two earlier annual progress reports (DOE/ER/60522-4 & -5) covered the first two years of the 3-year grant cycle. Because this is the last year of a 3-year grant cycle, this report will summarize progress over the entire 3-year period. The overall goals of the grant are to develop novel instrumentation and techniques for the performance of biological and materials research, and especially for the development of x-ray detectors suitable for use at storage ring sources. Research progress has been excellent and the overall goals, as well as most of the specific goals have been successfully met.

The research is well guided by the philosophy that useful instrumentation development and basic biostructural and materials research are inseparably coupled and proceed effectively when the instrumentation development is driven by specific needs of basic research problems. To insure this coupling, the Princeton Biophysics group has avoided the division of the group into "instrumentation" and "applications" specialists; rather, each group member is simultaneously involved in both development of instrumentation and the application of the instrumentation developed to basic research problems being worked on by that individual.

Because this is the last progress report in a 3-year grant cycle, it is useful to very briefly summarize the high points of the results over the entire three-year period. The period 1/1/90 thru the present has been one of fruition for the development of CCD-based area x-ray detectors, ancillary instrumentation and biomaterials research. By now, the Princeton Biophysics group has overseen the assembly, testing, and application of over a half dozen CCD-based detectors. The process has involved detailed evaluations of all the components (hardware and software) of these devices. Several of the detectors are now well out of the development stage and are routinely used to perform biomaterials research. Large scale development for dedicated storage ring application is now underway.

At the same time, significant biomaterials research has been performed with the detectors and ancillary instrumentation developed under this grant. Of particular significance are studies on the effects of high pressures on biomembranes and experiments on lipid-protein interactions.

With respect to the training goals of the grant, two graduate students have been trained through to a Ph.D., four undergraduates have graduated with senior theses performed on the apparatus, and two post-docs are part way through their training.

Specific accomplishments of the grant include:

- A) A prototype large area CCD x-ray detector was assembled and tested at CHESS. This led into development work on a dedicated detector based on a 2048 × 2048 CCD.
- B) Two new CCD-based x-ray detector designs, one based on a reducing image intensifier and one based on a fiber-optic taper, were assembled, tested and put into use.
- C) Ancillary x-ray detector components, including phosphor structures, intensifiers, fiber optics, software, CCDs and electronic components were designed, constructed, and/or tested.
- D) A comparative study of CCD detectors, image plates and film was performed.
- E) Work on pixel array detectors was initiated.
- F) Ancillary beamline and high-pressure instrumentation was developed for biomaterials characterization.
- G) Low-light level research was performed on color image intensification, lyoluminescence and luminescence during polymerization.
- H) Much biomembrane, biomaterials, and materials research was accomplished.

Reports of the accomplishments of the research performed under this grant for the 1/1/92-12/31/92 period are tabulated in section III, below, and have been published in 20 papers, 17 abstracts, 1 technical report, 4 undergraduate and two Ph.D theses; in addition, 4 research papers, derived from the theses are in various stages of preparation for submission. Copies of publications not submitted with earlier progress reports are attached. Capsule summaries of the major research accomplishments are given, below. The references listed below each subheading refer to papers and theses; consult the list of abstracts for abstract information.

II) RESEARCH ACCOMPLISHMENTS

A) Large Format CCD Detector

References: Eikenberry et al, 1991; 1992.

Eikenberry et al (1991; 1992) describe the assembly and evaluation of a prototype directly coupled large format (2048 × 2048) CCD detector for use at a storage ring source. The detector was specifically designed to meet the stiff requirements of protein Laue diffraction. It consisted of a thin phosphor coupled to a thin fiber optic plate, which was in turn liquid film coupled to an experimental CCD 55 mm across. The prototype was evaluated by applications testing at the CHESS facility at Cornell University. The results were quite spectacular: quantum limited operation was achieved simultaneously with spatial resolutions better than 50 um. The detector was shown to yield better quality data than contemporaneously acquired storage phosphor images. The tests unequivocally demonstrated that directly coupled large format CCD detectors are feasible and have the capability

of meeting some of the more difficult detector requirements encountered at storage ring sources.

The results of the tests were reported at several crystallographic meetings to a great degree of enthusiasm. This led to the formation of a collaborative effort between our group, CHESS, and others to develop a detector for use at CHESS based on a 2048 x 2048 CCD and a 3:1 fiber-optic taper (see the Supplemental Application dated Nov. 11, 1991 or the current renewal application, date May, 1992 for a complete description of the collaboration). This detector will serve as a test platform for similar devices intended for beamlines at the APS.

B) Other CCD Detector Designs

References: Tate (1991); Eikenberry et al, 1992; manuscripts in preparation.

Three other CCD detector designs were also carried through to completion, including assemble, testing, and installation for dedicated research use. The first design is a retrofit of the detector of Reynolds et al, 1978 (Rev. Sci. Instr. 49: 1241) by replacement of the slow-scan vidicon camera with a commercially available cooled, slow-scan CCD unit (Photometrics model CC-200). The result was a great improvement in overall performance, including a wider dynamic range, better resolution, less distortion, better stability, and fewer systematic effects. The detector allowed operation of the image intensifier in the system at a fraction of the gain needed with the old vidicon camera. Non-linearity problems in the A/D electronics were encountered with the Photometrics CC-200 200 Khz camera design which necessitated using a 50 Khz model. This detector is now in dedicated use in our laboratory and will be fully described in a manuscript in preparation. It is suitable for SAXS problems for which an appropriate image intensifier is available.

The second detector design consists of a custom fabricated 80 mm input area single stage zoom intensifier lens coupled to a TEK512 CCD. This detector has the advantage of a larger input area and at least two vendors who are willing to supply the image intensifier. It has the disadvantages of a compromised resolution when operated at sufficient reduction in the image tube to achieve high gain, quantum limited operation. Although the detector is quite suitable for use with diffuse signals, it was decided to try and improve the characteristics by operating with less image reduction in the intensifier and to perform the remaining reduction and image coupling via a reducing fiber-optic taper. The new configuration is now under assembly. The detector is intended to be used for polymer and membrane diffraction problems.

The final detector configuration consists of a single stage proximity focussed intensifier with a custom, low persistence phosphor coupled via a 5:1 fiber-optic taper to a fiber-optic input Thomson 512 x 512 detector. The CCD is controlled by a Princeton Scientific

Instruments GEN-V controller. This detector performs beautifully and is highly suitable for a wide variety of diffraction problems in which the input aperture is limited to about 70 mm on the diagonal. The detector will be fully described in a manuscript in preparation. It is now being routinely used for biomembrane research. This detector is highly suitable for storage ring applications in which the detector readout time of about 4 seconds is not limiting, ie, a large fraction of the static diffraction problems encountered. The detector is not count-rate limited even at the highest available count-rates.

C) Ancillary X-ray Detector Components

Reference: Deckman et al, 1990; manuscripts in preparation; Eikenberry et al, 1991.

Many problems involving intensifiers, fiber-optic components, phosphors, CCDs, and electronic CCD controllers were investigated over the course of assembling and evaluating the four CCD detectors described in sections A & B, above; these are discussed in detail in the papers describing the respective detectors. A few points are worth noting here: (1) Several phosphor deposition procedures were developed which have improved efficiency without substantially compromising the detector resolution. The trade-offs between the use of different phosphors and different deposition procedures were examined in the context of different experimental needs. (2) Fiber-optic taper technology appears to be such that very high quality tapers up to 75 mm diameter are available. Beyond that, the vendor base and the quality of the tapers both become severely limiting. (3) Image intensifiers and air-path optics are to be avoided, if possible, because of inevitable electron and light scatter problems, which compromise the ultimate contrast attainable. Image tubes are also suffering from a decreasing vendor base. (4) High quality CCD control electronics appear to be an art. Although many different controllers are available, few meet the stringent noise limitations required to fully utilize the capabilities of modern CCDs. (5) Trapless CCDs have only recently become available. This is important because directly coupled fiber-optic based detectors do not have airpaths suitable for the introduction of the "fat-zero" light biasing which has traditionally been used to fill traps prior to exposure.

D) Detector Comparisons

Reference: Eikenberry et al, 1992.

A comparative study was performed on the capabilities of CCD detectors, x-ray storage phosphors and x-ray film. The study, performed at Princeton and at CHESS demonstrate that storage phosphors lack the sensitivity of CCD detectors and have serious systematic problems (probably mostly due to light scattering in the optical paths of the image plate reader and in the plates) which compromise low contrast signal detection. In fact, at very low contrasts (e.g. 5-10%), film is a superior detector to storage phosphors. At higher

contrasts, and given sufficient signal, which is the case encountered with many traditional protein crystallographic problems, storage phosphors are very good detectors. In all cases, the CCD detectors delivered superior performance, but, of course, with a smaller sensitive area. The importance of this study is that it shows that the published evaluations of storage phosphors are optimistic and delimits the diffraction situations where storage phosphors should and should not be used.

The comparative evaluation was performed using the storage phosphor system installed at CHESS. It would be useful to redo this study with other storage phosphor systems, since the performance is a function of the image plate reader. A follow-up study using a Fuji reader is planned.

E) Pixel Array Detectors

Work on pixel array detectors was delayed when it became clear that serious development would require far greater resources than were available under the current DoE grant. This was unfortunate because pixel array detectors have the potential to solve the most difficult detector problems projected for the APS. An opportunity to begin serious research on pixel arrays was presented by the APS Collaborative Research Program. A proposal to jointly develop pixel arrays with the APS was submitted and accepted and now planning is moving along rapidly. More detail on this is given in the current grant renewal application dated May, 1992.

F) Ancillary Beamline and High Pressure Instrumentation

References: Strzalka, 1992; Tate et al, 1992; So et al, 1992; So, 1992; manuscripts in preparation.

Tate et al, 1992, describes apparatus for time-resolved diffraction and the application to a temperature-jump study of membrane phase transitions at the NSLS. There are substantial difficulties in obtaining uniform heating in bulk specimens; therefore, we investigated the possibilities of initiating events via pressure-jumps (P-jumps).

It turns out that biomembrane lipid mesomorphic transitions are exquisitely pressure sensitive. So et al (1992) describes automated pressure and temperature beamline apparatus for the performance of static x-ray diffraction. The Ph.D. thesis of Peter So (1992) is a detailed investigation of the structure and thermodynamics of lipid-water mesomorphic transitions vs. pressure and temperature. It includes the design, construction, and application of a novel dilatometer for measurement of the volume changes of small specimens vs. pressure; this data is used, via the Clausius-Clapeyron relation, to extract the thermodynamic potentials of the specimens. The thesis then goes on to describe a series of high pressure x-ray cells and their application to the study of the structure of lipid-water

dispersions under high pressures. Papers detailing the instrumentation and results of So (1992) are presently being written.

So (1992) forms the basis of a P-jump experiment scheduled for the NSLS in June, 1992.

G) Low-Light Level Research

References: Reynolds, 1990; 1991; 1992; Hajduk & Reynolds, 1992 (abstract).

Reynolds (1991) describes procedures which could be used to obtain color information in image intensified images. Normally, color information is lost because the image is incident upon a single photo-cathode and the output image is a phosphor. However, by appropriate spatial translations of images of different colors, spectral information may be obtained in simultaneous, parallel channels.

Reynolds (1990; 1992) describe research on lyoluminescent phenomena with an aim of understanding the effect and determining if it is suitable for luminescent dosimetry.

Hajduk & Reynolds (1992) reports on the observation of luminescence from polymerizing acrylamide gels. The source of luminescence is not understood, but it is speculated that it might be useful as a diagnostic for chemical reactions which occur upon polymerization.

H) Biomaterials and Materials Research

References: Boni et al, 1990; Kaufman et al, 1990; Lewis et al, 1990; Turner, 1990; Gruner & Shyamsunder, 1991; Narayan et al, 1990; Gruner, 1991; 1992; Turner et al, 1992a & b; Gawrisch et al, 1992; So, 1992; Polcyn, 1992.

As indicated in the Introduction section, every Biophysics group member is involved both in instrumentation development and in the use of the instrumentation for basic research. Briefly:

Gruner (1992) and Tate et al (1992) are invited reviews summarizing the biomembrane phase transition work carried out by the Princeton Biophysics group over the past 7 years.

Boni et al (1990) and Lewis et al, (1990) are characterization of synthetic lipid and surfactant molecules.

Gruner & Shyamsunder (1991) and Gruner (1991) describe how membrane physical properties may affect membrane protein activity. It is speculated that anaesthesia may be mediated via membrane elastic effects on proteins.

Kaufman et al (1990) describes a study of the physical and chemical properties of the membrane from a plasmalogen-deficient bacterium.

Turner (1990) and Turner et al (1992a & b) describe x-ray and neutron diffraction based studies on the structure of nonlamellar biomembrane lipid phases. Turner et al

(1992b) forms the basis of a neutron diffraction study of lipid chain statistics scheduled for the High Flux Beam Reactor at Brookhaven National Lab in the Fall of 1992.

Narayan et al (1990) describe a high pressure method for determining the change in overall volume upon hydrating a nearly fully-hydrated lyotropic liquid crystal. It tests the assumption of linear addition of water and lipid volume, which underlies a vast quantity of biomembrane literature. The assumption is shown to be valid.

So (1992) and Polcyn (1992) describe the effects of both positive and negative pressures upon biomembrane lipids, as characterized via high pressure x-ray diffraction and dilatometry.

III) PUBLICATIONS, THESES & REPORTS (1/1/90 - 6/1/92)

(Note: Papers not previously submitted with prior progress reports are included with this progress report as a separate package).

A) Papers & Technical Reports

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C) Theses

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Strzalka, Joseph W. (1991). Design and characterization of a capacitance-based dilatometer prototype (Senior Thesis, Princeton Elec. Engineering Dept.).

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MASTER

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of meeting some of the more difficult detector requirements encountered at storage ring sources.

The results of the tests were reported at several crystallographic meetings to a great degree of enthusiasm. This led to the formation of a collaborative effort between our group, CHESS, and others to develop a detector for use at CHESS based on a 2048 x 2048 CCD and a 3:1 fiber-optic taper (see the Supplemental Application dated Nov. 11, 1991 or the current renewal application, date May, 1992 for a complete description of the collaboration). This detector will serve as a test platform for similar devices intended for beamlines at the APS.

B) Other CCD Detector Designs

References: Tate (1991); Eikenberry et al, 1992; manuscripts in preparation.

Three other CCD detector designs were also carried through to completion, including assemble, testing, and installation for dedicated research use. The first design is a retrofit of the detector of Reynolds et al, 1978 (Rev. Sci. Instr. 49: 1241) by replacement of the slow-scan vidicon camera with a commercially available cooled, slow-scan CCD unit (Photometrics model CC-200). The result was a great improvement in overall performance, including a wider dynamic range, better resolution, less distortion, better stability, and fewer systematic effects. The detector allowed operation of the image intensifier in the system at a fraction of the gain needed with the old vidicon camera. Non-linearity problems in the A/D electronics were encountered with the Photometrics CC-200 200 Khz camera design which necessitated using a 50 Khz model. This detector is now in dedicated use in our laboratory and will be fully described in a manuscript in preparation. It is suitable for SAXS problems for which an appropriate image intensifier is available.

The second detector design consists of a custom fabricated 80 mm input area single stage zoom intensifier lens coupled to a TEK512 CCD. This detector has the advantage of a larger input area and at least two vendors who are willing to supply the image intensifier. It has the disadvantages of a compromised resolution when operated at sufficient reduction in the image tube to achieve high gain, quantum limited operation. Although the detector is quite suitable for use with diffuse signals, it was decided to try and improve the characteristics by operating with less image reduction in the intensifier and to perform the remaining reduction and image coupling via a reducing fiber-optic taper. The new configuration is now under assembly. The detector is intended to be used for polymer and membrane diffraction problems.

The final detector configuration consists of a single stage proximity focussed intensifier with a custom, low persistence phosphor coupled via a 5:1 fiber-optic taper to a fiber-optic input Thomson 512 x 512 detector. The CCD is controlled by a Princeton Scientific

Instruments GEN-V controller. This detector performs beautifully and is highly suitable for a wide variety of diffraction problems in which the input aperture is limited to about 70 mm on the diagonal. The detector will be fully described in a manuscript in preparation. It is now being routinely used for biomembrane research. This detector is highly suitable for storage ring applications in which the detector readout time of about 4 seconds is not limiting, ie, a large fraction of the static diffraction problems encountered. The detector is not count-rate limited even at the highest available count-rates.

C) Ancillary X-ray Detector Components

Reference: Deckman et al, 1990; manuscripts in preparation; Eikenberry et al, 1991.

Many problems involving intensifiers, fiber-optic components, phosphors, CCDs, and electronic CCD controllers were investigated over the course of assembling and evaluating the four CCD detectors described in sections A & B, above; these are discussed in detail in the papers describing the respective detectors. A few points are worth noting here: (1) Several phosphor deposition procedures were developed which have improved efficiency without substantially compromising the detector resolution. The trade-offs between the use of different phosphors and different deposition procedures were examined in the context of different experimental needs. (2) Fiber-optic taper technology appears to be such that very high quality tapers up to 75 mm diameter are available. Beyond that, the vendor base and the quality of the tapers both become severely limiting. (3) Image intensifiers and air-path optics are to be avoided, if possible, because of inevitable electron and light scatter problems, which compromise the ultimate contrast attainable. Image tubes are also suffering from a decreasing vendor base. (4) High quality CCD control electronics appear to be an art. Although many different controllers are available, few meet the stringent noise limitations required to fully utilize the capabilities of modern CCDs. (5) Trapless CCDs have only recently become available. This is important because directly coupled fiber-optic based detectors do not have airpaths suitable for the introduction of the "fat-zero" light biasing which has traditionally been used to fill traps prior to exposure.

D) Detector Comparisons

Reference: Eikenberry et al, 1992.

A comparative study was performed on the capabilities of CCD detectors, x-ray storage phosphors and x-ray film. The study, performed at Princeton and at CHESS demonstrate that storage phosphors lack the sensitivity of CCD detectors and have serious systematic problems (probably mostly due to light scattering in the optical paths of the image plate reader and in the plates) which compromise low contrast signal detection. In fact, at very low contrasts (e.g. 5-10%), film is a superior detector to storage phosphors. At higher

contrasts, and given sufficient signal, which is the case encountered with many traditional protein crystallographic problems, storage phosphors are very good detectors. In all cases, the CCD detectors delivered superior performance, but, of course, with a smaller sensitive area. The importance of this study is that it shows that the published evaluations of storage phosphors are optimistic and delimits the diffraction situations where storage phosphors should and should not be used.

The comparative evaluation was performed using the storage phosphor system installed at CHESS. It would be useful to redo this study with other storage phosphor systems, since the performance is a function of the image plate reader. A follow-up study using a Fuji reader is planned.

E) Pixel Array Detectors

Work on pixel array detectors was delayed when it became clear that serious development would require far greater resources than were available under the current DoE grant. This was unfortunate because pixel array detectors have the potential to solve the most difficult detector problems projected for the APS. An opportunity to begin serious research on pixel arrays was presented by the APS Collaborative Research Program. A proposal to jointly develop pixel arrays with the APS was submitted and accepted and now planning is moving along rapidly. More detail on this is given in the current grant renewal application dated May, 1992.

F) Ancillary Beamline and High Pressure Instrumentation

References: Strzalka, 1992; Tate et al, 1992; So et al, 1992; So, 1992; manuscripts in preparation.

Tate et al, 1992, describes apparatus for time-resolved diffraction and the application to a temperature-jump study of membrane phase transitions at the NSLS. There are substantial difficulties in obtaining uniform heating in bulk specimens; therefore, we investigated the possibilities of initiating events via pressure-jumps (P-jumps).

It turns out that biomembrane lipid mesomorphic transitions are exquisitely pressure sensitive. So et al (1992) describes automated pressure and temperature beamline apparatus for the performance of static x-ray diffraction. The Ph.D. thesis of Peter So (1992) is a detailed investigation of the structure and thermodynamics of lipid-water mesomorphic transitions vs. pressure and temperature. It includes the design, construction, and application of a novel dilatometer for measurement of the volume changes of small specimens vs. pressure; this data is used, via the Clausius-Clapeyron relation, to extract the thermodynamic potentials of the specimens. The thesis then goes on to describe a series of high pressure x-ray cells and their application to the study of the structure of lipid-water

dispersions under high pressures. Papers detailing the instrumentation and results of So (1992) are presently being written.

So (1992) forms the basis of a P-jump experiment scheduled for the NSLS in June, 1992.

G) Low-Light Level Research

References: Reynolds, 1990; 1991; 1992; Hajduk & Reynolds, 1992 (abstract).

Reynolds (1991) describes procedures which could be used to obtain color information in image intensified images. Normally, color information is lost because the image is incident upon a single photo-cathode and the output image is a phosphor. However, by appropriate spatial translations of images of different colors, spectral information may be obtained in simultaneous, parallel channels.

Reynolds (1990; 1992) describe research on lyoluminescent phenomena with an aim of understanding the effect and determining if it is suitable for luminescent dosimetry.

Hajduk & Reynolds (1992) reports on the observation of luminescence from polymerizing acrylamide gels. The source of luminescence is not understood, but it is speculated that it might be useful as a diagnostic for chemical reactions which occur upon polymerization.

H) Biomaterials and Materials Research

References: Boni et al, 1990; Kaufman et al, 1990; Lewis et al, 1990; Turner, 1990; Gruner & Shyamsunder, 1991; Narayan et al, 1990; Gruner, 1991; 1992; Turner et al, 1992a & b; Gawrisch et al, 1992; So, 1992; Polcyn, 1992.

As indicated in the Introduction section, every Biophysics group member is involved both in instrumentation development and in the use of the instrumentation for basic research. Briefly:

Gruner (1992) and Tate et al (1992) are invited reviews summarizing the biomembrane phase transition work carried out by the Princeton Biophysics group over the past 7 years.

Boni et al (1990) and Lewis et al, (1990) are characterization of synthetic lipid and surfactant molecules.

Gruner & Shyamsunder (1991) and Gruner (1991) describe how membrane physical properties may affect membrane protein activity. It is speculated that anaesthesia may be mediated via membrane elastic effects on proteins.

Kaufman et al (1990) describes a study of the physical and chemical properties of the membrane from a plasmalogen-deficient bacterium.

Turner (1990) and Turner et al (1992a & b) describe x-ray and neutron diffraction based studies on the structure of nonlamellar biomembrane lipid phases. Turner et al

(1992b) forms the basis of a neutron diffraction study of lipid chain statistics scheduled for the High Flux Beam Reactor at Brookhaven National Lab in the Fall of 1992.

Narayan et al (1990) describe a high pressure method for determining the change in overall volume upon hydrating a nearly fully-hydrated lyotropic liquid crystal. It tests the assumption of linear addition of water and lipid volume, which underlies a vast quantity of biomembrane literature. The assumption is shown to be valid.

So (1992) and Polcyn (1992) describe the effects of both positive and negative pressures upon biomembrane lipids, as characterized via high pressure x-ray diffraction and dilatometry.

III) PUBLICATIONS, THESES & REPORTS (1/1/90 - 6/1/92)

(Note: Papers not previously submitted with prior progress reports are included with this progress report as a separate package).

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