DEVELOPMENT AND COMMERCIALIZATION OF A NOVEL SYSTEM TO ASSESS INTRA-OPERATIVE GRAFT PATENCY DURING CORONARY ARTERY BYPASS GRAFT SURGERY

BY

GURPREET S. MANGAT

A Thesis submitted to
the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

Department of Pharmacology and Therapeutics
University of Manitoba
Winnipeg, Manitoba

The Faculty of Graduate Studies
500 University Centre, University of Manitoba
Winnipeg, Manitoba R3T 2N2

Phone: (204) 474 9377
Fax: (204) 474 7553
graduate_studies@umanitoba.ca

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

DEVELOPMENT AND COMMERCIALIZATION OF A NOVEL SYSTEM TO ASSESS INTRA-OPERATIVE GRAFT PATENCY DURING CORONARY ARTERY BYPASS GRAFT SURGERY

BY

GURPREET S. MANGAT

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

Of

DOCTOR OF PHILOSOPHY

TABLE OF CONTENTS

FIGURES & TABLES

Abbreviations

CABG	Coronary artery bypass graft
ICG	Indocyanine green
NIR	Near infrared
CCAC	Canadian Council on Animal Care
ATP	Acceptance test plan
EMC	Electromagnetic compatibility
OR	Operating room
FDA	Food and Drug Administration
HPB	Health Protection Branch
LIMA	Left internal mammary artery
RIMA	Right internal mammary artery
OM	Obtuse marginal
LAD	Left anterior descending
RCA	Right coronary artery
PDA	Posterior descending artery
CX	Circumflex
RCA	Right coronary artery
CCD	Charge coupled device
SRA	System risk analysis
CK	Creatinine kinase
CK-MB	Creatinine myoglobin
EKG	Electrocardiogram

LDH	Lactate dehyrogenase
CD	Compact disc
AST	Aspartate aminotransferase
ALT	Alanine transaminase

Acknowledgements

I would like to thank my supervisor Dr. John Docherty who has provided on-going support, encouragement and mentorship. His patience, inspiring nature and assistance in removing barriers have allowed me to transform an idea from a bench top experiment to a clinical product that is benefiting thousand of patients worldwide. It has been a true honor to have worked with him.

In addition the on-going support and encouragement of the following individuals is especially recognized:

Dr. Grant Hatch, Dr. Wayne Lautt & Dr. Ian Smith:Thesis committee
Dr. Hong Tian, Dr. Roxanne Deslauriers, Ms. Bo Xiang, Ms. Bozena Kuzio & Ms. Shelley Germscheid: National Research Council of Canada, Institute for Biodiagnostics.
Dr. Robert Flower who has assisted in answering my numerous questions over the years.
Dr. Gary Glavin: University of Manitoba.

I thank my wife for being supportive throughout this period and of course my mother whose on-going support of my education has allowed me to achieve many of my current successes.

"In loving memory of my father Mr. Parminder Singh Mangat, a teacher, a friend and my mentor"

Abstract

The research forming the basis of this thesis began through a study designed to study the effects of various pharmacological agents on vasculature in animal models. In order to achieve this goal a system was developed that would allow researchers to image vessels using fluorescence angiography.

The angiography involves using a dye, indocyanine green (ICG) which has been used in the clinical setting for over 40 years to study cardiac output, hepatic function and choroidal blood flow in the eye. The initial work was performed on murine femoral arteries and subsequently studies were completed on coronary vessels in the porcine model. The resulting angiograms were of very high quality with respect to their qualitative data. However, quantitative analysis of vessel diameter proved to be a much more arduous task. The software utilized to perform the function of calculating vessel diameter is effective in the static environment where there is no motion of the vessel; however, in the in vivo setting the ability to register images so as to allow the tracking of vessels over time was not possible. As such the initial hypothesis that such a system could be used in vivo to study the effects of agents on vessel diameter was not successfully proven.

The angiograms generated however, were studied and it was determined that such a system could potentially be invaluable if it could be used in the surgical setting for determining graft patency during coronary artery bypass graft (CABG) surgery in humans. Approximately one million patients undergo CABG surgery on an annual basis. The focus, thus shifted to adapt the system for this purpose

and subsequently a system was developed that could be used in the operating room that would allow cardiac surgeons to obtain real time intra-operative angiograms demonstrating whether the grafts they had just constructed were patent.

The impact of the technology in initial human studies performed was significant. It was found that in 5-8% of cases graft revision was required based on the angiograms. These angiograms allowed the surgeons to correct the defects prior to closing the patient, thereby, improving the efficacy of the treatment. The tremendous interest in the system subsequently lead to endeavors to commercialize the technology in which regulatory approval was sought in Canada, Europe, Japan and the United States in order that the system could be marketed. In addition, studies were continued at multiple sites in the United Kingdom, Japan, Italy, Switzerland and Canada to demonstrate efficacy and safety.

The system has been used to date in over 1000 patients worldwide and a company, Novadaq Technologies has been formed to commercialize the technology and develop further applications.

CHAPTER I

INTRODUCTION

Fluorescence imaging has seen a tremendous growth in both the research and clinical environments. Advances in hardware technology have allowed researchers to exploit the chemical properties of agents that, to date, have been untapped. It is in this context that the research in this thesis has been developed. Initially, the objective of this thesis was to develop an angiographic procedure to study the effects of various pharmacological agents on the myocardial vasculature. The angiographic technique developed allowed the generation of angiograms that provided excellent qualitatative data but unfortunately did not permit the quantitative analysis of the data. In hindsight the requirements initially set out were most likely too optimistic. However, as will be described, the system developed has proven to be invaluable in the clinical setting during cardiac bypass graft surgery. The thesis will outline the numerous steps that have been undertaken over a five year period whereby a study that was initiated in the laboratory has led to a commercial product that is currently being used worldwide. The focus will be on the initial animal studies performed to develop the technique for studying the effects of pharmacological agents and the subsequent animal and human studies to assess the scientific properties of the product that have led to its success in the clinical setting. In developing this product an encompassing scientific approach has been taken whereby expertise in the fields of pharmacology, biochemistry, physiology and electrical, mechanical and computer engineering have been utilized.

It is intended that by reading this thesis the reader will acquire a clear sense of the complexities and requirements for transforming a laboratory bench-top

project into a commercial product. The product, which is used for bypass graft patency confirmation, is outlined in the following chapters. However, it should be noted that it is a combination product in that it involves a chemical agent (indocyanine green dye (ICG)) and a device. This area is a rapidly growing field, which presents numerous challenges, especially with regards to regulatory requirements, and exciting opportunities. The objective is that this thesis can be used as a reference for the processes required for commercialization of a device. It is recognized that while this thesis will not encompass all areas of medical device development, the main principals are relevant to a large majority of combination products. The six areas of focus for the thesis are:

(1) Device development

(2) Device description

(3) Drug pharmacology

(4) Clinical data

(5) Regulatory requirements

(6) Further applications and conclusions

Although there are numerous further areas that have led to this product's success (e.g. pre-marketing studies, financing etc), they are beyond the scope of this thesis. However, it is envisioned that one day these elements can be combined with this document to give a complete story of the journey of an idea to a successful commercial product.

The research behind the development of the device stems from work that was initiated to study the effects of pharmaceutical agents on the coronary

vasculature. Studies were initiated in the animal model to demonstrate that the change in diameter of coronary arteries could be measured using an indocyanine green dye based fluorescence technique. The initial studies will be outlined in chapter II. However, it should be noted that the goal of developing such a technique was not successful but led to the determination that such an imaging modality could be used during coronary artery bypass graft surgery for the determination of graft patency. This identification of the clinical need came about as the data were presented at meetings whereby clinical interest stemmed from the extraordinary images created in the small animal model which allowed identification of vessels as small as 50 microns. Clinicians were consulted as to their interest in determining graft patency during coronary artery bypass graft surgery and the resounding positive response led to a focus on developing the device for this application.

This thesis commences below with an outline of the clinical need for such a device in order to first put in perspective what the goals were for this research project. Following this introduction there is a review of the six areas of interest mentioned above. The document concludes with the author's thoughts on the process and the many challenges that have been faced. It is hoped that this "story" is both interesting and informative.

Clinical Need

Stenotic atherosclerotic coronary artery disease is a narrowing of the coronary arteries, caused by atherosclerosis that limits the blood flow to the myocardium. In its most severe form it causes total occlusion of the coronary artery. The

disease is most often diagnosed after a patient presents with symptoms of angina. Contrast X ray angiography performed in the catheterization lab provides the definitive diagnosis.

In this procedure the cardiologist catheterizes target coronary arteries, accessed through the femoral or radial artery, allowing selective delivery of contrast agent to the artery of interest. Currently there are three main treatment options for coronary artery disease; medical treatment based on drug therapy, percutaneous angioplasty performed by interventional cardiologists or coronary artery bypass graft (CABG) surgery (1).

The efficiency of angioplasty procedures has increased dramatically following the introduction of stents to keep the artery open (2). However, the biological response to the foreign nature of the stent implanted into the human body has resulted in a high rate of restenosis in and around the stent. In many cases this has necessitated a repeat revascularization procedure, either by repeat stenting or by surgery. In large part much of the problem with stent restenosis appears to have been solved with the recent introduction of drug eluting stents (3). Evidence for the long term benefits of the drug eluting stents is not presently available and awaits the results of suitable long term trials. For many patients CABG surgery remains the treatment of choice for coronary artery disease (4).

CABG surgery employs the use of a vascular graft (e.g. saphenous vein or internal mammary artery) that forms an alternate conduit for blood supply to the myocardial tissue. In 1995 over 15,000 of these procedures were performed in Canada (5) with an additional one million surgeries being performed worldwide

(estimated). The success of this procedure, in terms of clinical improvement, depends on short and long-term graft patency. Graft occlusion in the first few months post operatively is thought to be due to poor blood flow, poor coronary arterial runoff, injury to the graft during preparation or faulty surgical technique (6). Studies to date have reported occlusion rates of 8-12% perioperatively (7, 8, 9, 10).The occlusion rate for saphenous vein grafts is 5% -20% during the first year and an overall occlusion rate of 22-30% at 5 years postoperatively (6). Use of the internal mammary artery has increased patency rates to 95% at one year, 94% at 8 years and 85% at 10 years (6). Intraoperative graft failure is a major cause of cardiac mortality and morbidity (11). Graft failure is a common cause of perioperative myocardial infarction and can be seen in up to 9% of CABG patients (11).

Recent advances in cardiac surgery have in large part focused on strategies to minimize deleterious effects to the myocardium e.g. by performing surgery on the beating heart. Although CABG without the use of cardiopulmonary bypass has been practiced for the past 10 years its acceptance has not been universal because of concerns about the quality of the anastomoses (12). Similarly, attempts have been made to minimize the invasiveness of the procedure leading to faster recovery, shorter hospital stay and cost reductions. There is increasing use of minimally invasive techniques for revascularization of the left anterior descending artery with a left internal mammary artery (13). There have however been even more concerns raised regarding the quality of the anastomoses based on the ability to perform an accurate anastomosis on a beating heart through a

limited access incision (14). With the advent of new stabilization tools which reduce motion in the area of the anastamosis to be performed, surgeons have become more adept at the procedure. However, a need for validation still exists. In a recent review of the literature on this topic (15) it was stated " Although meaningful comparison is not possible, it can probably be concluded that early angiographic graft patency by either conventional or MIDCABG technique is generally 90% or greater". In view of the large numbers of these procedures that are performed any increase in this early patency rate translates into a large decrease in the number of patients who suffer the ill effects of a less than perfect graft.

It would therefore appear that there is a definite need for a reliable means to test the patency of anastomoses at the time of surgery. Angiography is widely accepted as the gold standard for assessing the quality of anastomoses. However, it is invasive, costly, time consuming and rarely available in the operating room. There have been recommendations for routine angiography early in the postoperative period (16) but this may be rather late in the process for prompt corrective action to be taken. Postoperative angiography, used to assess graft patency before hospital discharge, has resulted in re-operation in some patients (17, 18).

Methods for Graft Assessment Currently Available

The gold standard for graft assessment is conventional coronary angiography (19), which can be performed intraoperatively using movable fluoroscopy equipment. However, this procedure is highly invasive, requiring arterial

puncture, is very time consuming and cumbersome and thus has not been accepted into general practice. Conventional contrast x-ray angiography can be performed in the catherization lab soon after surgery. This procedure requires the transfer of a critically ill patient, and also necessitates the administration of x-ray contrast agents, which are nephrotoxic, and conceivably of greater risk immediately following open heart surgery, as surgery is associated with renal dysfunction. For these reasons this test is generally reserved for those patients exhibiting ischemic changes suggestive of an acute graft occlusion.

Several other methods have been developed and employed for intraoperative assessment of graft patency. These have included electromagnetic (20), ultrasound flow measurement (21), Doppler velocity waveform (22), epicardial echocardiography (23) and thermal coronary angiography (24). All of these techniques have limitations with respect to resolution and flow assessment resulting in difficulty confirming graft assessment.

In light of these limitations the objective of this thesis was to develop a fluorescence based imaging system which can be used safely and effectively in the operating room to detect graft patency. The system that has been developed combines both a device (SPY™ System) and a pharmaceutical agent (indocyanine green dye). Indocyanine Green fluorescence imaging offers the potential for a reliable, non invasive, inexpensive and rapid method of intraoperative assessment of graft function. With such a tool, surgeons would be able to check the quality of their procedures and revise, redo or perform additional procedures as dictated by the images.

Currently the SPY System is being used in clinics in the United Kingdom, Switzerland, Italy, Japan and Canada and most recently has been introduced into 2 sites in the United States (Stanford University Hospital and Medical City, Dallas)

CHAPTER II

Effects of pharmacological agents on vessel diameter in the mouse femoral artery and porcine coronary vasculature

Initial pharmacological studies performed in the mouse and porcine animal models

Background

It was the initial intent of my work to develop an imaging technique that would allow researchers to study the effects of pharmaceutical agents on the vasculature. Specifically it was intended that the methodology would provide a means to calculate the change in vessel diameter of vessels when exposed to varying concentrations of agents. The initial work was performed on a mouse model whereby I intended to study the effects of acetylcholine on the femoral artery. Following determination that the effects of acetylcholine could be determined in the mouse model (femoral artery) a study was commenced to determine the change in coronary vasculature under the influence of a multitude of agents in the porcine model. The two studies are outlined below along with a summary of the results and the conclusions.

Effects of acetylcholine on mouse femoral artery diameter (n=6)

The mouse was prepared by inducing anesthesia in an induction box using isoflurane. (Ohmeda Pharmaceutical Products, Mississauga, On, Canada) (4% in medical air, 4L/min) and maintained by use of a facemask providing isoflurane at a rate of 1.5-2.0% in medical air (400ml/min). During the experiment, the mouse was positioned on a thermostatted water blanket, with body temperature being monitored by a rectal temperature probe. To facilitate imaging of the vessels of interest, the thoracic, abdominal and inguinal areas of the mouse were shaved, the mouse positioned on its back, and the skin over the femoral vasculature was resected to expose the vasculature of interest. The jugular vein was cannulated

using a piece of stretched PE10 tubing filled with saline containing 50 U heparin/ml.

After the mouse was prepared, a 10ul bolus IV injection of ICG was administered, followed by an IV injection of 50ul of saline solution. To prepare the bolus, 4ug/ml of clinical grade ICG was dissolved in sterile aqueous solvent within one hour of injection. All injections were administered via the cannula established in the jugular vein. The saline was used to flush the line and to ensure passage of an intact bolus through the femoral vasculature, producing a sharp wavefront.

The angiographic images were collected using a KP-160 video camera (Hitachi Denshi Ltd, Tokyo, Japan). The KP-160 camera was selected because it is highly sensitive in the near-infrared region of the electromagnetic spectrum (which is also where ICG fluoresces), thus optimizing the capture of light emitted from the excited ICG. An 845nm bandpass filter (845DF25), Omega Optical Inc., Brattleboro, VT) was coupled to the camera to exclude all photons that were not of the wavelength associated with ICG fluorescence.

The laser diode used to excite the ICG was positioned at a 45 degree angle to the area of investigation in order to minimize specular reflectance (i.e. glare) arising from surface water. Glare is a major source of visual noise during imaging. The laser device included a SDL-820 laser diode driver (SDL Inc., San Jose, CA) that maintained a continuous wave output with an average current of 3.95A). The laser diode was used to illuminate the area of interest and excite the ICG dye, thereby inducing fluorescence in the region being imaged. A laser diode was used because, unlike an incandescent light source, a laser emits photons in a

narrow frequency range, and thus eliminates the need for an excitation filter and the associated problem of heat dissipation. Because the laser-emitted wavelengths are limited, the excitation filter can be eliminated, improving the fluorescence. Consequently, a higher proportion of the light emitted from the laser diode is of the wavelength absorbed by ICG. It was found that use of an 800nm bandpass filter (800DF20, Omega Optical, Brattleboro, VT) in conjunction with the laser light source improved the results by selectively passing photons emitted at 806nm.

An analog to digital converter (752x480 pixel, 8 bit image processor, Model PIXCI-SV4, Epix Inc., Bffalo Grove, IL) was employed to digitize the composite video signal output from the camera. Image analysis was performed using XCAP for Windows 95/98/NT version 1.0 (Epix Inc., Buffalo Grove, IL). The image processing algorithm included the following steps.

(1) Selection of vessels of interest. The anatomy of the vasculature varies between animals. Consequently, it was necessary to develop criteria for the selection of an area of interest. This process began with the positioning of the camera. The camera was positioned so that the field of view included the femoral artery and its branches. For purposes of image analysis, the vessels of interest were the femoral artery and the branches that provided the highest resolution and the greatest degree of branching, usually tertiary or quaternary.

(2) Calibration. The positioning of the camera with respect to the area being imaged varied with each animal, and it was therefore necessary to

calibrate the camera for every image collected. A small diameter (320 um) capillary tube (TSP320450; Polymer Technologies, LLC, Phoenix, AZ) filled with ICG was used to calibrate the images. The image processing software includes a built-in calibration function that allows the specification of a set of pixel co-ordinates and the assignment of a user defined value of the distance between the coordinates. The software's edge detector was used to determine the co-ordinates of the edges of the dye fluorescing in the capillary tube. The inner diameter of the capillary tube, in microns, was then assigned to the length of the distance between these points. Because this is a built-in feature of the software, all subsequent measurements in all frames of the image were stated in microns, rather than pixel units. To avoid distortions due to camera movement or other stochastic phenomena, every image was calibrated. The advantages of this technique are that the same method was used to measure the calibration device as was used to measure the vessel, and the calibration device is measured in the same frame under the same optical conditions as the vessels.

(3) Measurement of diameter using sub-pixel edger. All vessel diameters were measured using the built-in sub-pixel edger.

(4) Selection of frames based on edge strength. Analysis of ICG images entails the selection of frames for analysis. The need to select frames is a consequence of the fast rate of ICG flow through the femoral artery with respect to the rate of image acquisition. This results in a leading and

trailing sequence of frames that were acquired before and after ICG were detectable in the area being imaged. Edge strength, which is automatically calculated by the edge detector in the software, is a measure of the relative strength of the edge, i.e., the ratio of the value of pixels on one side of the edge to the value of those on the other side. The ratio is highest when the contrast is greatest, which corresponds to the greatest intensity of ICG fluorescence. The vessels that were measured have two edges, thus ten frames in which the product of the edge strengths was the greatest were selected for analysis . After the foregoing was completed, the vessel diameters and standard errors were calculated as described above. Students t-test for paired values was applied to determine the statistical significance between measurements (border of significance, p=0.01)

Data on the effects of different size vessels in the mouse femoral artery are given in the table below.

	Vessel Diameter (microns)				
Acetylcholine Concentration	Control	0.01 uM	0.1 uM	1.0 uM	10.0 uM
Primary	92.7±1.2	58.2 ± 1.3	61.5 ± 1.7	58.3 ± 1.5	64.6 ± 1.5
Secondary	69.4 ± 0.3	67.0 ± 1.3	75.1 ± 1.2	90.0 ± 1.8	75.0 ± 1.4
Tertiary	57.5 ± 0.7	42.9 ± 0.6	44.9 ± 0.6	47.1 ± 1.2	42.9 ± 0.8

The foregoing demonstrates the ability of the system to observe the flow of blood through a static vessel, to determine the diameter of that vessel and to monitor changes in the reactivity of the vessel after the administration of acetylcholine.

The system worked well with respect to the ability to accurately measure the diameter of vessels as seen with the baseline measurement of 3 sizes of vessel. However, the results obtained in response to application of acetylcholine are the opposite to those anticipated. The acetylcholine was applied topically to the exposed vascular bed to minimize system wide effects. It was anticipated that this approach would yield a robust dilation of the vessels. Interestingly since this work was completed similar studies have been performed using Orthogonal Polarization Spectral (OPS) imaging, which yields high-contrast images of vascular beds by visualizing red blood cells (25). In this study the authors report up to 199% vasodilation of the saphenous artery in response to topically applied acetylcholine. In that study the exposed vasculature was continuously superfused with warm physiological saline and drugs added to the superfusate. In this study attempts were made to ensure that the drug was applied at physiological temperature but it is likely that the temperature was somewhat below physiological and the observed results are due to cold mediated vasoconstriction rather than direct effects of the acetylcholine. Nonetheless the experiment does demonstrate the proof of principle of the technique. However, in order to study the effects of agents in a physiological setting such as the coronary vasculature it was required that the system be capable of determining vessel diameter change where the target is in flux/motion. As such experiments were performed using ICG angiography to study the effects of pharmacological agents on the porcine coronary vasculature.

Indocyanine Green Cardiac Angiography to study the effects of pharmacological agents on the porcine coronary vasculature

Background

The study of the effects of drugs on the coronary vasculature has been an exciting field of research over the last two decades as front line physicians and surgeons are increasing their reliance on pharmaceuticals to treat cardiovascular disease. In general, vasoactive drugs have the potential to act at several sites (26,27,28). Thus, they can act on arterial vascular beds, venous beds or a combination of both. In addition arterial vasodilators may demonstrate relative selectivity for small (resistance) or large (conduit) vessels. This selectivity in site of action may be important in determining the appropriate choice of drug to help remedy a disease specific perfusion deficit. The question then arises as to how best to asses the site of action of vasoactive compounds. To date animal studies have focused on studying the effects of these compounds on coronary vessels *in vitro* (26,28,29). The in vitro models have provided valuable data, however, it is recognized that these techniques have their limitations since the *in vivo* responses may be markedly different due to the abundance of internal systems controlling the vascular response (for example the perfusion pressure experienced by the coronaries during perfusion in situ with the heart performing physiologically relevant workloads). In the study we hoped to address these limitations in that we planned to study the effect of various compounds on the

coronary vasculature in an open chest porcine model. We intended to show that the indocyanine green cardiac imaging system could be used to determine changes in vascular responses in vessels ranging from 40 microns to 300 microns in diameter.

Hypothesis:

Indocyanine Green Cardiac Angiography can be used to study the vascular response of coronary vessels of various size (40-300 microns) *in vivo*, to pharmacological agents in the pig.

Objectives:

(1) Assess the ability of indocyanine green cardiac angiography to produce angiograms which can be digitized and analyzed using edge detection software.

(2) Develop a calibration system to determine vessel diameters.

(3) Test pharmacological agents known to cause coronary vasculature responses in order to determine the sensitivity of the system.

Indocyanine green angiography has been used extensively for over 30 years in performing retinal angiography and the dye has been used for assessing cardiac and hepatic output in humans (30). As such, the toxicity data are readily available (31,32,33,34,35) and there are no adverse effects which would indicate any harm to the animal.

The choice of pharmacological agents to test was based on compounds which are used extensively in clinical practice to treat conditions such as angina.

Pharmacological agents to be tested:

(1) Nitroglycerin (1.2-11ng/ml): A vasodilator that acts by donating NO (nitric Oxide)

(2) Nifedipine (47 ug/ml): A Calcium channel blocker with selectivity for coronary arteries

(3) Bradykinin (60-600 ng over 1 minute): An endogenous compound that causes vasodilation. Levels of bradykinin are likely to be increased during treatment with an angiotensin converting enzyme (ACE) inhibitor.

(4) Acetylcholine (0.1-1000 Nm): An endogenous substance that causes the release of NO from endothelial cells and thus causes vasodilation.

(5) Sodium Nitroprusside (0.1nM-1uM): An agent similar in action to nitroglycerin but has more pronounced effects on the venous vessels.

(6) Serotonin (1-1000nm): An endogenous substance that causes vessels to constrict.

The ability to confirm that the technique is capable of accurately identifying changes in vessel diameter to pharmacological agents would provide an invaluable tool for researchers and pharmaceutical companies in the future to assess new compounds *in vivo*. Furthermore, we would be able to determine if these drugs have differential effects on vessels of varying diameter and as such have preferential indications in the treatment of cardiac perfusion deficiencies. The experimental protocol is summarized below.

Experimental protocol

Domestic pigs (n=8) were obtained two weeks prior to use. The animals were fasted overnight before the day of the experiment. Atropine (0.5 mg/kg body wt) was given intra-muscularly 30-50 minutes prior to the experiment. Anesthesia was induced with an intra-muscular injection of Midazolam (0.4 mg/kg body wt) and ketamine (20 mg/kg body wt). Isoflurane in 30-35% O_2 (by mixing medical air and pure oxygen), 3% initially, was given by mask and maintained at 1.5-3% following trachea intubation. The depth of anesthesia was controlled at the level at which the animal does not show spontaneous breath or muscular response to surgical procedures with a mean blood pressure of 60-80 mmHg by adjusting the level of isoflurane. The minimum level of isoflurane was not allowed to be less than 1.5 %. The right carotid artery was cannulated for arterial pressure monitoring and blood sampling. A sternotomy was performed and the pericardium was opened longitudinally along the midline. A 0.035" cannula was inserted into a carotid artery and advanced until it just entered into the LAD in order to administer drugs directly to the site of action in the coronary vasculature. Placement of the cannula was confirmed by touch and also by imaging a dye placed within the lumen of the cannula.

Cardiac imaging was performed prior to and following the administration of the drugs using the ICG imaging system. A bolus of ICG (5mg/ml) was injected through a peripheral vein and its passage through the coronary vasculature monitored and recorded by the imaging system. This was accomplished using a system which evenly illuminates the heart with light from a 806 nm laser (to

cause the ICG to fluoresce as it passes through the heart). The device also incorporates a CCD camera, equipped with suitable filters, to acquire images (at the rate of 60/s) of the fluorescent dye as it passes through the heart.

In order to determine the vessel diameter, a tube of known diameter and length (and filled with dye) was placed in the field of view (sutured to the pericardium) to act as a calibration standard.

Drugs were infused directly into the LAD and all measurements were made on this vessel and its branches. The drug was infused at the appropriate concentration and then washed out from the heart to the peripheral vasculature where it was diluted and underwent distribution about the body. It was therefore possible to study a number of drugs , over a range of concentrations, in each animal.

Drugs were infused for a period of one minute, with image acquisition occurring over the period 40s to 60s. Five minutes was allowed between each infusion of drug to allow for dilution and distribution of the drug in the peripheral vasculature.

Drugs were administered in the following order;

1. Acetylcholine (0.1-100nM)
2. Bradykinin (60-600ng over 1 minute))
3. Nitroglycerin (1.2-11ng/ml)
4. Nifedipine (47ug/ml)
5. Sodium Nitroprusside (0.1nM-1uM)
6. Serotonin (1-100nM)

Data were analyzed using commercial software from Epix Inc. (Sub-pixel edge detection system). It was then hoped that dose response curves (% change in vessel diameter vs drug dose) could be constructed for each drug for vessels of various size (eg LAD, 1st order branch, 2nd order etc). At the end of the study, the animal was euthanized under general anesthesia by infusion of a high potassium solution and exsanguination.

Results & Conclusions

The ability to image the coronary vessels and obtain clear high resolution angiograms was accomplished. However, the next step which entailed using edge detection methodology to determine vessel diameter proved to be a much more arduous task. I was not able to consistently track the vessels on the moving heart using the commercially available software. Numerous interactions with the manufacturer of the software (Epix Inc., Buffalo Grove, Illinois) did not resolve the issue that the edge detection tools could not accurately remain locked on the vessel over a short period of time during which the pharmacological agents were acting. Consultation with software experts at the National research Council of Canada (Institute for Biodiagnostics, Informatics Group) led to the realization that a specific software package would be required that could register each image to the previous image in order to follow the edge of the corresponding vessel. The experts in this area were not confident that such a modality could be developed due to the inherent problems in registering beating heart images captured at a frame rate of 30 frames per second.

The results were very disappointing given the excellent quality of the angiograms but it had to be accepted that the ability to quantify the change in diameter was not feasible at the time.

As a part of the interaction with imaging experts (basic researchers and clinicians) to resolve these issues it became apparent that even if the images could not be used to quantify changes in blood flow their qualitative angiographic detail could be used in certain clinical settings. The most obvious need was identified to be in the use of the system to determine graft patency during coronary artery bypass graft surgery. This would not require quantitative assessment but rather only high resolution angiograms that could show the flow of blood through grafts and distal coronary beds. As such development began to modify the system for this application and test it initially in the porcine model using the lessons learned to date followed by use in humans. The following chapter outlines the development of the system and studies undertaken and lead by me to determine if such a system could be produced.

CHAPTER III

DEVICE DEVELOPMENT

In developing the device for use in the cardiac operating room (OR) the following requirements had to be met:

(i) Ease of use: due to the complexity of the CABG procedure itself, the introduction of any additional procedures such as intra-operative imaging could only be adopted if they can

a. be performed by the existing staff in the operating room

b. be completed in approximately fifteen minutes or less in order not to lengthen the total procedural time significantly

c. provide results immediately for review.

(ii) Ease of interpretation: the images should be intuitively easy to interpret with little or no post-acquisition data processing.

(iii) Inexpensive: due to cost restraints in all areas of the health care system, the procedure must not add more than $500 per case.

(iv) Safety:

a. the device must be safe for both the patient and the staff in the operating room. Ideally, no protective equipment would be required, unlike the need for lead aprons in situations where ionizing radiation is used.

b. Imaging agents used must have a proven safety profile in humans.

(v) Mobile: the device had to be transportable between Operating Rooms since most hospitals have multiple OR's and as the device is not required throughout the procedure it could be shared among operating rooms, thereby allowing for cost savings through multiple usage.

(vi) Sterile field compatible: since the surgical field is sterile, the device must have some type of sterile wrap or ability to be sterilized in order that it can be placed in the sterile field.

The development of the SPY system occurred over a period of 4 years. The process involved taking early data from bench top experiments (Figure I) and combining it with feedback from clinicians to develop a system that could be used in the operating room. Further revisions were then made as feedback from clinical use was studied. The process can be outlined by the following seven steps:

(1) Animal studies: proof of principle

(2) Intellectual property registration

(3) Determination of device specifications

(4) System risk analysis (SRA)

(5) Manufacturing plan

(6) Validation

(7) Safety testing

In order to understand the process and the complexities involved, each of these areas will be discussed in further detail with special attention being paid to summarizing the early animal work that formed the foundation of the final product.

1. Animal Studies

In order to initiate the animal work a prototype was developed for animal use and is shown in Figure II. The initial prototype had the following key characteristics:

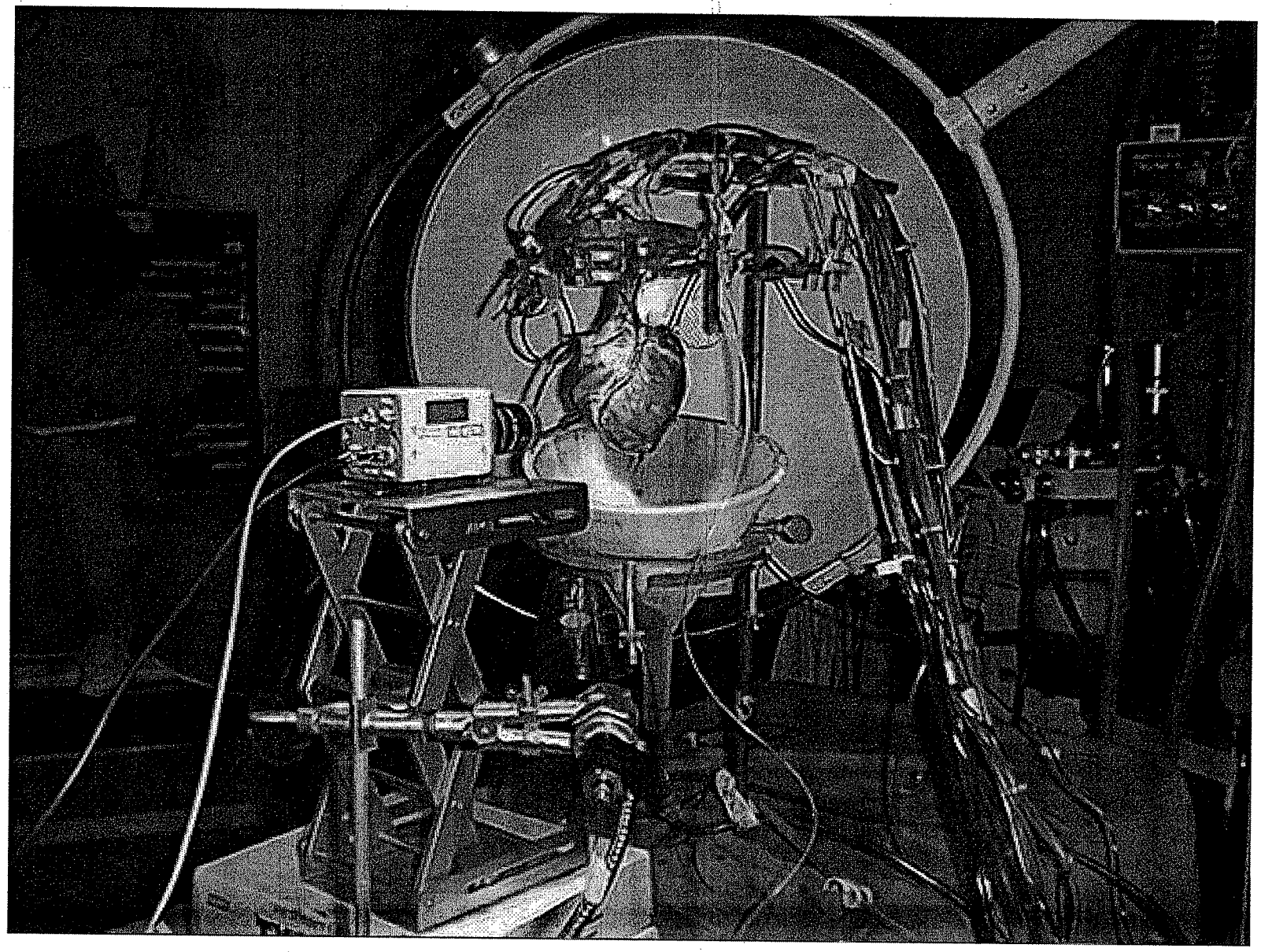


Figure I: Bench-top experiment (porcine heart)

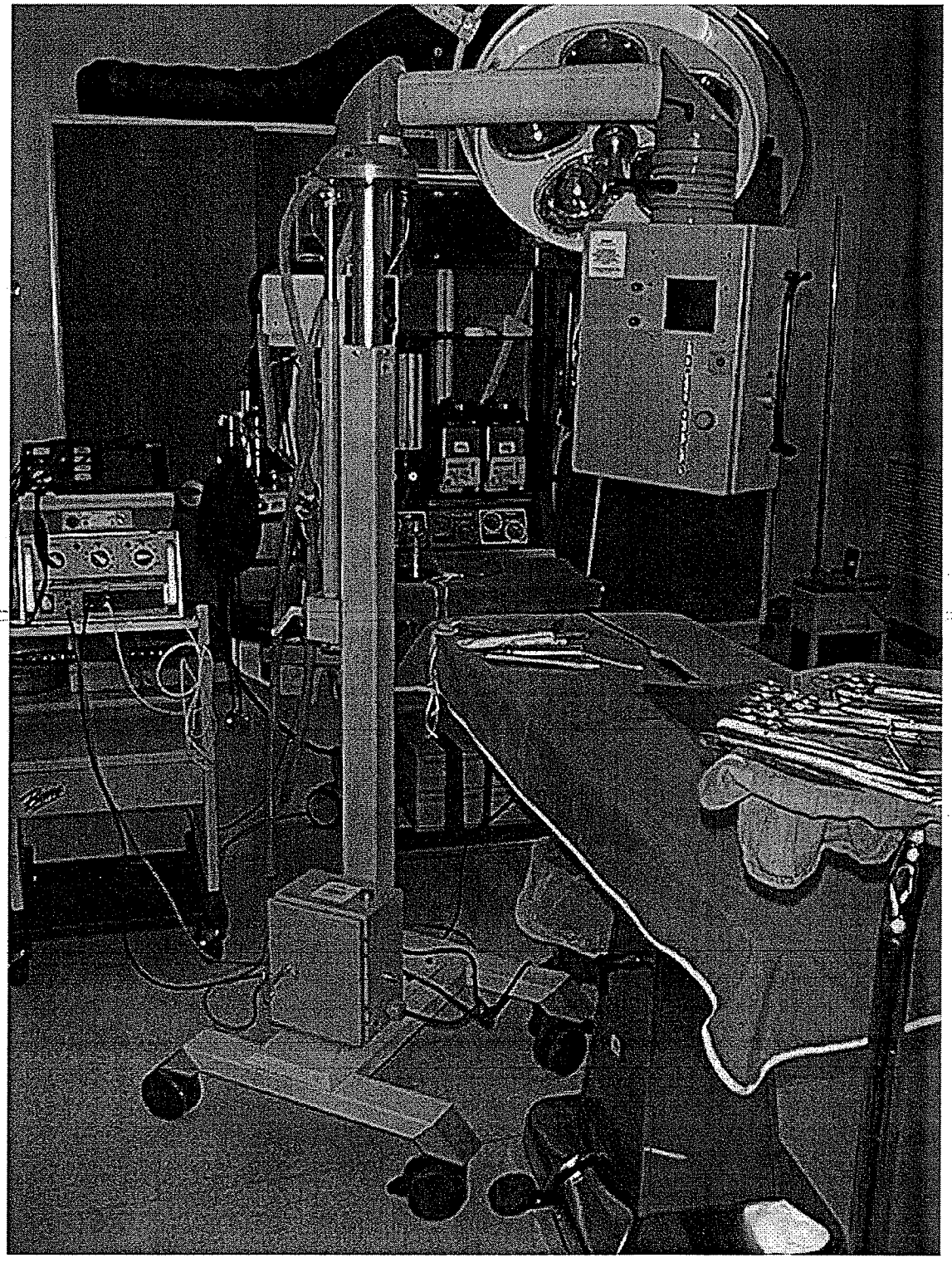


Figure II: Prototype developed for animal work

(i) CCD camera: for image capture. Camera selected on basis of high sensitivity in near infrared region of spectrum.

(ii) Laser light source: single laser light source at 806nm – ICG absorbs maximally at 806nm. The light was altered from a single beam point source to a uniform illumination grid (approx. 3" x 3") using optical lenses. This grid was required in order that the entire surface of the heart could be illuminated.

(iii) High resolution monitor and super very high speed (SVHS) recorder: to allow for immediate review of the image sequence and recording.

The following studies were performed in order to demonstrate a proof of principle for the imaging technology in the animal model:

(i) Establishment of imaging parameters (n=6)

(ii) Imaging of coronary artery bypass graft surgery in the porcine model (n=6)

(iii) Finalizing of imaging parameters (n=6)

(iv) Imaging the posterior aspects of the heart (n=6)

The studies and their respective results are outlined below:

(i) Establishing imaging parameters

Objectives

These studies were designed to establish the imaging parameters prior to initiating studies on the coronary artery bypass graft model in pigs. The overall objective was to ensure that it would be possible to acquire high quality images

of the coronary vasculature at the time of bypass graft surgery. Specific objectives necessary to achieve this overall objective were:

1. To determine the concentration of dye solution for bolus injection.
2. To determine the volume of dye solution to be administered.
3. To determine if a saline flush is necessary following the bolus injection of dye.
4. To determine if administration of the dye into a peripheral vein yields high quality images of the coronary vasculature.
5. To determine if the 3W laser provided sufficient illumination for imaging the pig heart vasculature in situ.
6. To determine if the optical configuration (i.e. filters) was appropriate for acquiring images of the pig heart vasculature.

Methods

A female mixed breed pig (35 kg) was obtained from a local breeder and acclimatized to the animal facilities for two weeks prior to use. The pig was fasted overnight prior to the experiment. The pig was sedated/premedicated with an intra-muscular injection of midazolam (0.4mg/kg), atropine (0.05 mg/kg) and ketamine (20 mg/kg) and transported from the animal holding facility to the surgical suite. Anesthesia was induced with isoflurane (4% in 30-35% O_2 obtained by mixing pure oxygen with medical air) delivered by mask. The pig was then intubated and anesthesia maintained with isoflurane (1.5-2% in 30-35% O_2) delivered through a positive pressure ventilator. The urinary bladder was cannulated for collection of urine throughout the procedure (to prevent leakage of urine during the procedure, not for measurement of urinary metabolites etc).

Vascular access was established in an ear vein for continuous delivery of saline. A cannula was inserted into the left femoral vein for delivery of dye and the left femoral artery was cannulated for measurement of arterial pressures and heart rate.

The chest was opened by means of a median sternotomy and a pericardial cradle constructed to support the heart. For acquisition of fluorescence coronary angiograms the prototype device was positioned over the heart and correct focus verified by examination of the image acquired in the presence of strong visible light.

ICG was initially administered at a concentration of 0.5 mg/ml and then at 5 mg/ml. Volumes of the bolus varied from 125 to 500 μl with and without a wash in flush of saline (2.5 ml).

Results and Discussion

Very weak fluorescence was observed when ICG was administered at a concentration of 0.5 mg/ml and it was readily apparent that a significant increase in the dye concentration was required. The weak signal was identified as a low fluorescent output within the coronaries providing very little contrast between the coronary vessels and the myocardial tissue. The concentration of dye was therefore increased to 5 mg/ml. At this concentration, a bolus of 0.25 to 0.5 ml gave excellent quality images (see Figure 2). The quality of these images was not further improved by washing in the bolus with a flush of saline. Administration of the dye bolus through a peripheral vein (the femoral for convenience in the pig) provided excellent images.

Figure III: Images of the coronary vasculature in the *in situ pig heart*

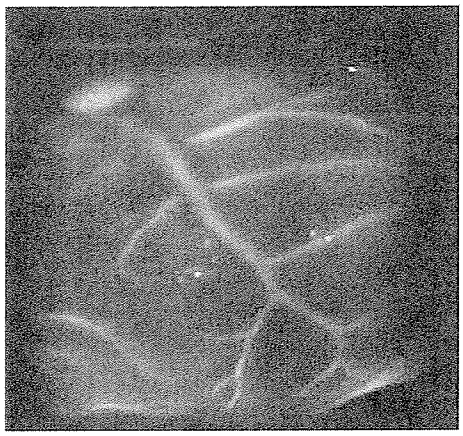


Figure IIIA

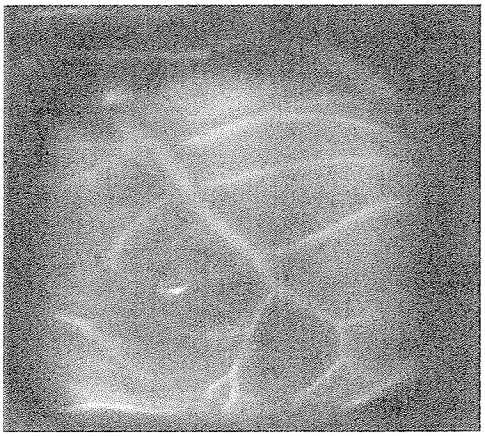


Figure IIIB

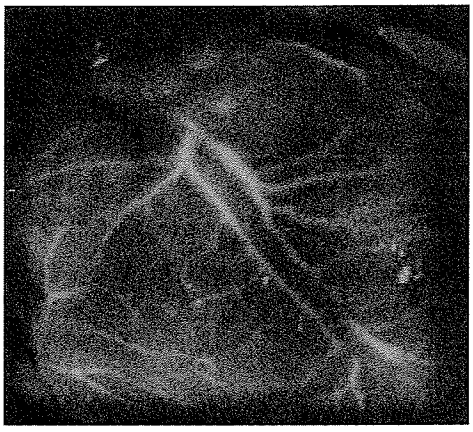


Figure IIIC

Figure IIIA shows the early phase of filling, with fluorescent dye, of the coronary arteries of a pig heart. Note the lack of signal from the myocardium. Figure IIIB shows a later phase of arterial filling but now there is a distinct "blush" to the myocardium indicating the presence of dye within the microvasculature. This blush may be taken as an indication of good nutritional blood flow. In Figure IIIC the dye can be seen filling the venous vasculature in the heart.

The specifications for construction of the prototype device were based on preliminary experiments on intact mice and isolated blood perfused pig hearts described in Chapter II. These experiments were performed using a stand- alone camera and laser. The prototype to be used in the CABG experiments essentially put these components together in an easy to use configuration. The specifications for the laser, filter and area of illumination were all appropriate for acquisition of high quality images of the porcine coronary vasculature in situ.

Conclusions

It was possible to acquire high quality images of the porcine coronary vasculature in vivo in a simple, reproducible manner using small volumes of ICG at a concentration within the limits of the current labeling for the use of this dye in humans. It was reasonable to initiate the studies on the porcine model of coronary artery bypass graft surgery to demonstrate the utility of the technique under these conditions.

(ii) Imaging of coronary artery bypass graft surgery in the porcine model

Background

The pig heart bears strong similarity to the human heart with respect to size, anatomy, function and vascular anatomy. For these reasons the pig offers an ideal model for validating procedures relating to human cardiac surgery. We chose the pig as the large animal model to validate indocyanine green fluorescence angiography as an adjunct to coronary artery bypass graft (CABG) surgery. The surgery was performed by a board certified cardiac surgeon

assisted by a surgery resident. Training in the appropriate perfusion techniques was provided to our animal health technicians by a perfusionist.

Methods

Surgery

CABG was performed on eight domestic pigs (68-74 kg, male and female) by standard techniques.

The pigs were prepared for surgery described in Section I (Establishing imaging parameters)

The chest was opened by means of a median sternotomy and a pericardial cradle constructed to support the heart. The left internal mammary artery (LIMA) was dissected free from the chest wall but was not skeletonized.

Cardiopulmonary bypass was established following cannulation of the ascending aorta and right atrium. The aorta was cross clamped and the heart arrested by delivery of 1 to 1.5 l cold cardioplegia (St Thomas':blood, 1:4) delivered through a cardioplegia cannula inserted in the ascending aorta. An anastomosis was then constructed between the left internal mammary artery and the left anterior descending (LAD) coronary artery using 7-0 polypropylene suture. The aortic cross clamp was removed and the pig weaned from bypass. As the pig heart is more susceptible to fibrillation than is the human heart, successful weaning from bypass usually required electrical defibrillation. Upon completion of the experiment the pig was euthanized by cardiac arrest following delivery of a bolus of KCl directly to the left ventricle.

In 4 animals flow down the conduit was measured by means of a doppler flow probe (1.5 mm, Transonics) placed on the LIMA. In instances where placement of the flow probe was proving troublesome this was not pursued for fear of compromising the conduit.

Fluorescence angiography

Prior to initiating the series of experiments involving the CABG procedure, one open chest pig was used in order to optimize the image acquisition parameters (see Establishing Imaging Parameters) and these parameters were then used in the subsequent experiments.

For acquisition of fluorescence coronary angiograms the prototype device was positioned over the heart and correct focus verified by examination of the image acquired in the presence of strong visible light. The laser was activated, the VCR set to record and a bolus of ICG (0.5 ml of a 5 mg/ml solution in sterile diluent) rapidly injected into the femoral vein. Passage of the fluorescing dye through the coronary vasculature was observed in real time on the video monitor and the laser deactivated when the dye had cleared the coronary vasculature. Angiograms were acquired prior to the CABG procedure and also at the end of the procedure following weaning from the bypass pump. A series of angiograms were acquired at the conclusion of the CABG procedure. Thus, an angiogram was acquired when both the LIMA and LAD were fully patent. A suture was then placed around the LAD proximal to the anastomosis, the LAD occluded and a further angiogram acquired. With the native flow through the LAD still restricted, the LIMA was then partially occluded and a further angiogram acquired. In two

animals angiograms were also acquired prior to weaning from the bypass pump (i.e. upon removal of the aortic cross clamp but prior to decannulating). In these instances the dye was delivered directly into the arterial line of the bypass circuit.

Results

Surgical Outcomes

One pig died on the surgery table due to technical difficulties encountered in the course of cannulating for cardiopulmonary bypass (the aortic anatomy of the pig is drastically different from that in the human and can cause problems for first attempts at cannulation). In 6 CABG procedures the results appeared excellent while in one procedure a thrombus occluded the anastomotic site.

Angiographic results

Baseline angiograms

Prior to performing the CABG procedures the acquisition of baseline angiograms demonstrated, as expected, excellent perfusion over the entire left ventricle. Soon (6-8 seconds) after injection of the dye into the femoral vein a diffuse signal was observed as the dye entered the left ventricular cavity and began to fluoresce. This strong fluorescence signal (from the large volume of blood in the left ventricle) was apparent through the left ventricular wall. Thereafter, the dye was observed in the coronary arteries, filling first the large vessels and then the smaller branches. The fluorescence then appeared as a background "flush" as the dye entered the arterioles and capillaries and then the venous vasculature could be visualized. The dye was then cleared from the coronary vasculature with some residual background fluorescence remaining for the next few minutes.

If required, repeat angiograms could be acquired immediately with no loss of resolution from the residual background. This sequence of arterial, capillary and venous filling gave an excellent visual indication that all areas of the heart were receiving adequate nutritional blood flow (see Figure III).

Post CABG angiograms

In 6 cases the post CABG angiograms indicated excellent perfusion over the entire left ventricle. In one procedure a thrombus caused occlusion at the anastomosis and this resulted in a serious perfusion deficit to a large area of the left ventricle. This was readily apparent on the fluorescence angiogram (the heart function was surprisingly good considering the extent of the perfusion abnormality).

When the LAD was occluded in the 6 cases mentioned above the initial image sequences were quite similar to those described below(see Figure IV). However, in this case there was a delay in perfusion of the LAD bed. Filling of the vascular beds other than those supplied by the LAD could be observed but with little apparent filling of the LAD region. Flow was observed in the LAD region shortly thereafter as the dye traveled down the LIMA and the area distal to the anastomosis was fully perfused. It should be pointed out that occlusion of the LAD did cause an increase in flow (as measured by doppler flow probe) down the LIMA, as might have been predicted.

When the LIMA was partially occluded (in addition to occlusion of the LAD) there was an obvious perfusion deficit in the LAD territory as both the native LAD flow and the LIMA conduit flow were compromised.

Angiograms acquired prior to weaning from cardiopulmonary bypass

Excellent image quality was obtained when the angiography was performed prior to weaning from bypass. The logistics involved in acquiring these images were also straightforward. Thus, the dye was delivered into the arterial filter by means of the associated stopcock with excellent results. The dye was also delivered into the arterial line by prior modification of the line by inserting a stopcock directly into the line. In both instances the dye delivery resulted in a sharp bolus of dye reaching the heart and resulting in a sharp wavefront passing through the coronary vasculature.

No attempts were made to perform quantitative analyses on the fluorescence angiograms but rather a qualitative assessment was made with respect to the overall quality of perfusion of the myocardium. This determination was made in part based on the difficulty of quantifying the data from the images as seen in the pig experiments described in chapter II. Upon review of the images it was readily apparent that the required information i.e. vessel patency could be identified from the qualitative angiogram alone. This lack of post acquisition data processing also meets one of the system requirements for a clinically useful device, an image that is intuitively simple to interpret. The image in Figure IV clearly demonstrates the ability of the system to provide high quality angiograms that can detect the lack of blood flow through a coronary artery or graft.

Figure IV – Fluorescent Angiogram of an In Situ pig heart following occlusion of the native coronary and bypass graft

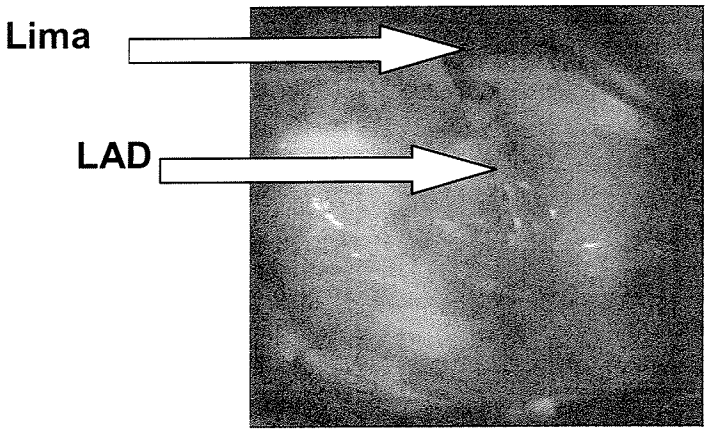


Figure IV shows a pig heart that has been subjected to a coronary artery bypass graft procedure involving grafting of the left internal mammary artery (LIMA) to the left anterior descending (LAD) coronary artery. Prior to acquiring this image the LAD was occluded proximal to the anastomosis and the LIMA was occluded. As expected the LAD was not visualized upon injecting the dye and there was no blush in the area of myocardium perfused by the LAD, indicating a lack of nutritional flow in this region.

Conclusions

The prototype device proved to be reliable and easy to use in an environment appropriate for performing CABG. High quality images were obtained reproducibly with little or no manipulation of the device. Once a pre-procedure series of angiograms had been acquired and the focal distance set it was a

simple matter to move the device adjacent to the table for acquisition of post-procedure angiograms. Thus, set up time for acquisition of each series of images was only 2-3 minutes. Angiograms acquired post-procedure demonstrated excellent perfusion of the myocardium. Disturbances to this perfusion pattern, resulting from selective occlusion of vessels were readily observed in the sequences of angiograms. Repeat angiograms were possible with little or no delay necessary between repeated acquisitions.

(iii) Finalizing the imaging parameters

Background

All of the imaging experiments to date had been performed using a Hitachi KPM2 RU camera and had yielded very high quality images. It was noted that Hitachi made an alternate version of this camera, the KPM2 RN, which is 10 times more sensitive in the 800 nm region (as determined experimentally). With this increased sensitivity available, studies were undertaken to determine if it is possible to decrease the laser power and thus the power density on the myocardial surface. The contrast agent, ICG, was being used at a concentration consistent with its current labeling. Given the long history of safe use of this compound (see Chapter IV), at total doses far exceeding the current application, it was not felt to be worthwhile to attempt to decrease the concentration of the dye. On the other hand, decreasing the laser power may help to allay whatever concerns there may be regarding safety issues with respect to the use of the laser in this application. One of the major concerns about the use of the laser is

the potential for it to cause thermal damage to the myocardial surface. A second objective of this study was to determine the effect of laser illumination on the myocardial temperature 1-2 mm from the surface.

Methods

A female mixed breed pig (65 kg) was obtained from a local breeder and acclimatized to the animal facilities for two weeks prior to use. The pig was fasted overnight prior to the experiment. The pig was prepared for surgery as described previously in Section I. The chest was opened by means of a median sternotomy and a pericardial cradle constructed to support the heart. For acquisition of fluorescence coronary angiograms the prototype device was positioned over the heart and correct focus verified by examination of the image acquired in the presence of visible light.

A thermocouple, 25G, was implanted 1-2 mm into the myocardium within the field of illumination of the laser and the temperature continuously monitored.

Body temperature was monitored using a rectal temperature probe.

Imaging Protocol

For each imaging sequence 0.5 ml of ICG dye (5 mg/ml) was delivered through the femoral vein. Following positioning of the device over the heart, the device was powered up and upon observing that the laser was activated (a few seconds after depressing the power button to allow a "soft start" to the laser) the dye was administered. Passage of the dye through the coronary vasculature was observed in real time on the monitor. The laser power button was kept depressed until the dye had cleared through the coronary venous system and in all cases the laser

was powered on for a minimum of 30 seconds. The temperature of the myocardium was observed throughout the illumination period and the highest reading recorded.

Baseline images were obtained using the KPM2 RU camera under the same conditions used in the previous studies i.e. full laser output (resulting in power densities of ~ 48 mWatts/cm^2 on the myocardial surface) and the lens aperture fully open (f stop 1.2). Subsequent imaging sequences were performed using the KPM2 RN camera at various laser outputs. The laser does not have a calibrated controller for varying the output and so the output was determined from the measured power density at a defined distance from the source. The power density was measure using an Orion laser power/energy monitor. Image sequences were acquired at laser outputs of 2.25, 1.5 and 0.75 Watts. At each laser output, images were acquired at three lens aperture settings; full (f stop 1.2), intermediate (f stop 2) and minimum (f stop 6). Images were collected directly to VHS tape and assessed visually.

Results and Discussion

Effects of laser illumination on temperature.

At the start of the experiment, body temperature was 38.2 °C and this drifted down to 37.5 °C over the course of the experiment. Laser illumination of the heart for imaging had no effect on body temperature. At the highest laser output (2.25 Watts) the temperature of the myocardium (at a depth of 1-2 mm) increased insignificantly from 38.00 ± 0.07 °C to 38.14 ± 0.09 °C (n = 5, mean ± SD). At 1.5 watts the myocardial temperature was 37.70 ± 0.10 °C before illumination and

37.73 ± 0.05 °C at the end of illumination. At 0.75 Watts, the pre and post temperatures were 37.60 ± 0.00 °C and 37.63 ± 0.06 °C respectively (for the latter two data sets n = 3 in each case). It is therefore apparent that thermal injury to the myocardium is not of concern at these laser outputs and durations of illumination.

Effect of laser output on image quality.

Using the KPM2 RN camera and the "standard" imaging parameters i.e. 2.25 Watts laser output and a fully open aperture (f stop 1.2) the fluorescence signal saturated the CCD causing a "whiteout" on the screen. Adequate images could be obtained using this camera and laser output only with the aperture stopped down to *f* 6. At this aperture setting, the visible image was not distinct making it difficult to confirm the focus and field of view in the visual image of the heart. At the lower laser outputs of 1.5 and 0.75 Watts, images acquired at aperture 1.2 also showed excessive "blooming" due to the presence of too much signal. Optimal image quality was observed at laser outputs of 0.75 to 1.5 watts using an intermediate aperture of 2, which also resulted in visible images bright enough to permit confirmation of focus and selection of field of view.

Conclusions

Use of the Hitachi KPM2 RN camera allows the laser output to be decreased to 0.75 to 1.5 Watts. It is likely best to aim for an output close to the 1.5 Watts, or equivalent to a power density of approximately 30 mWatts/cm^2 on the myocardium. At these outputs, the camera could be operated at an aperture setting that yielded visible images bright enough to be used for confirmation of

focus and field of view selection. In addition, at these power levels, there was no effect on the myocardial temperature.

(iv) Imaging the posterior aspect of the heart

Background

All of the imaging experiments to date have concentrated on the anterior portion of the heart with visualization of the left anterior descending (LAD) coronary vascular bed. In a clinical setting it will be desirable to also image the vascular beds on the posterior portion of the heart. There are two possible approaches to address this problem. The first is to have a mirror or lens that can be inserted under the heart to reflect images back and up to the imaging device. This would be technically quite challenging and, in addition, the feedback from surgeons suggests that they would not be keen on introducing yet another element into the surgical field. The second approach is to physically elevate the heart and image the posterior of the heart. This is the approach that has been unanimously recommended by surgeons that have been consulted on this issue. In fact, this manipulation is performed on a routine basis during beating heart surgery. In the present study, use was made of a device that has been developed to maintain the heart in such a position for an extended period of time during beating heart surgery- the Achieve system with the Xpose positioning device from Guidant.

Methods

A female mixed breed pig (60 kg) was obtained from a local breeder and acclimatized to the animal facilities for two weeks prior to use. The pig was fasted

overnight prior to the experiment and prepared for surgery as previously described in Section I.

The chest was opened by means of a median sternotomy and the Guidant Achieve system used to retract the open edges of the sternum. When its use was necessary, the Xpose device (Guidant) was attached to the Achieve system. The Xpose device is a suction device incorporating a cup that attaches to the apex of the heart and allows the apex of the heart to be lifted out of the chest cavity. When used in conjunction with the Achieve system, it permits positioning of the heart for construction of anastomoses on the posterior aspect of the heart.

Fluorescence images of the coronary vasculature were acquired following positioning of the prototype device over the heart. The imaging head of the device was angled to achieve optimal visualization of the appropriate vascular beds and the Xpose system was used for stabilization of the heart during imaging of the posterior wall. ICG was administered as a bolus (0.5 ml of 5 mg/ml in sterile diluent) into the left femoral vein. Images were acquired using the prototype device with the slight modification of using a 16 mm lens rather than the 12 mm used in the previous studies. With this modification, the area illuminated by the laser occupied a larger proportion of the total field of view, in effect conferring a slight degree of magnification to the system. In addition, as images were acquired with the heart in a vertical position for some of the image sequences, it was necessary to maintain focus over the entire length of the heart. To achieve this effect advantage was taken of the increased sensitivity of the model KPM2 RN camera

to close down the aperture of the lens to *f* 12. The laser power for image acquisition was set at 2.25 Watts.

Images were acquired of the left anterior descending coronary artery, the posterior intraventricular branch, the left circumflex and the right coronary artery.

Results and Discussion

Visualization of all of the coronary beds was possible, using the Xpose system as required. The manipulations required to visualize each of the vascular beds were well tolerated with the mean arterial pressure decreasing from 67 mm Hg to 53-56 mmHg

High quality images of each of the vascular beds were acquired using the prototype imaging device.

It is worth noting that although in some instances the heart was in effect standing on end pointing upwards and the camera was focussed on the mid point of the heart, it was still possible to keep the entire heart in focus and acquire excellent images of the entire vascular bed on the myocardial wall being interrogated. It is further worth noting that despite the deep position of the heart within the porcine chest cavity it was possible to visualize, and image, the entire vascular bed on each aspect of the heart.

Conclusions

Excellent images of all coronary vascular beds were acquired from the in situ porcine heart. This was achieved using a relatively unsophisticated prototype device with limited degrees of freedom of movement. The acquisition of similar

images, from patients, using a more advanced imaging device having greater flexibility and degrees of freedom of movement is thus entirely feasible.

It was possible to keep the entire heart in focus, even with the heart in a vertical position, by making use of the superior sensitivity of the KPM2 RN camera to work at a lens aperture of *f* 12 when operating the laser at 2.25 Watts.

2. Intellectual Property

Having determined that the methodology had the potential for use in a commercial setting, it was imperative that a patent be filed in order to protect both the invention of the device and furthermore, the methodology for its use. This documentation would be essential in the commercialization process. As such, the following patent was developed with assistance from a patent attorney (Leydig Voit and Mayer, Chicago, USA): "Method and apparatus for performing intra-operative angiography" (Docherty, Mangat, Flower, Hewko, Chari). Initially a Provisional Patent was filed in order to secure a priority date while a full patent application was being developed. The full patent application was filed with the US and European patent offices within a year of filing the provisional patent.

3. System Requirements

In order to progress with a device which could be used in the clinical setting it was necessary to develop a clear set of specifications which could be used not only to develop the device but also test against once the device was complete. The following broad-based specifications for the system were determined based upon experience from the animal studies and pre-clinical feedback (cardiac surgeon advisors).

(a) Must produce high quality digital images that can be saved on CD/DVD

(b) Should not add greater than 15 minutes to the bypass procedure

(c) Must be mobile

(d) Operation should not require personnel that are not routinely available in the operating suite.

(e) Safety apparel should not be required for patient or operating room staff (e.g. laser safety glasses)

4. System risk analysis (SRA)

In developing any medical device it is essential that all potential risks are identified and mitigated if possible. It is not always possible to make changes to completely mitigate a risk factor, however an understanding that it exists can be used to reduce the likelihood of its occurrence through such actions as training or addition of safety indicators e.g. warning labels or lights. As such the development of a detailed system risk analysis was undertaken and the document is attached as Appendix B. This document is under continuous revision as new risks are identified and mitigated where possible.

5. Manufacturing

Following the development of the specifications and SRA the process of manufacturing the test devices for clinical trials was commenced. The arduous process of finding a suitable group to assist in this process will not be outlined. However, it should be understood that this process is where the results of a pharmacology/physiology study have to be coordinated with mechanical, optical and electrical engineering. The process involved close work with the engineers to

insure that the principles derived during the animal studies were incorporated into the device. This collaboration over an eight month time period led to the development of the SPY 1000™ system. The drawings for the device (mechanical and electrical) will not be included since they are beyond the scope of this thesis. Figures V and VI show the SPY 1000™ and the upgraded SPY 2000™ system which is currently available commercially.

6. Validation

Following the completion of the initial system, it was then imperative that an adequate test procedure be developed in order to verify and validate the manufacturing process. A validation test protocol (Acceptance Test Plan: ATP) was developed for the manufacturer (Automation Tooling Systems, Cambridge, Ontario) in accordance with their internal quality system requirements. This is the test procedure now used for all similar devices manufactured. It should be noted that the ATP is revised as revisions are made to the device or based on data coming back from use in the field.

7. Safety

With respect to safety of the device the work performed is outlined below:

(i) Electrical safety testing: performed by an outside body (TUV) to test for leakage current etc.

(ii) Electromagnetic compatibility (EMC) testing: performed by an outside body (TUV Rheinland, Germany)

(iii) Animal tests: to insure that the interaction of the laser with the myocardium and ICG do not result in any adverse events.

Figure V: SPY 1000 System

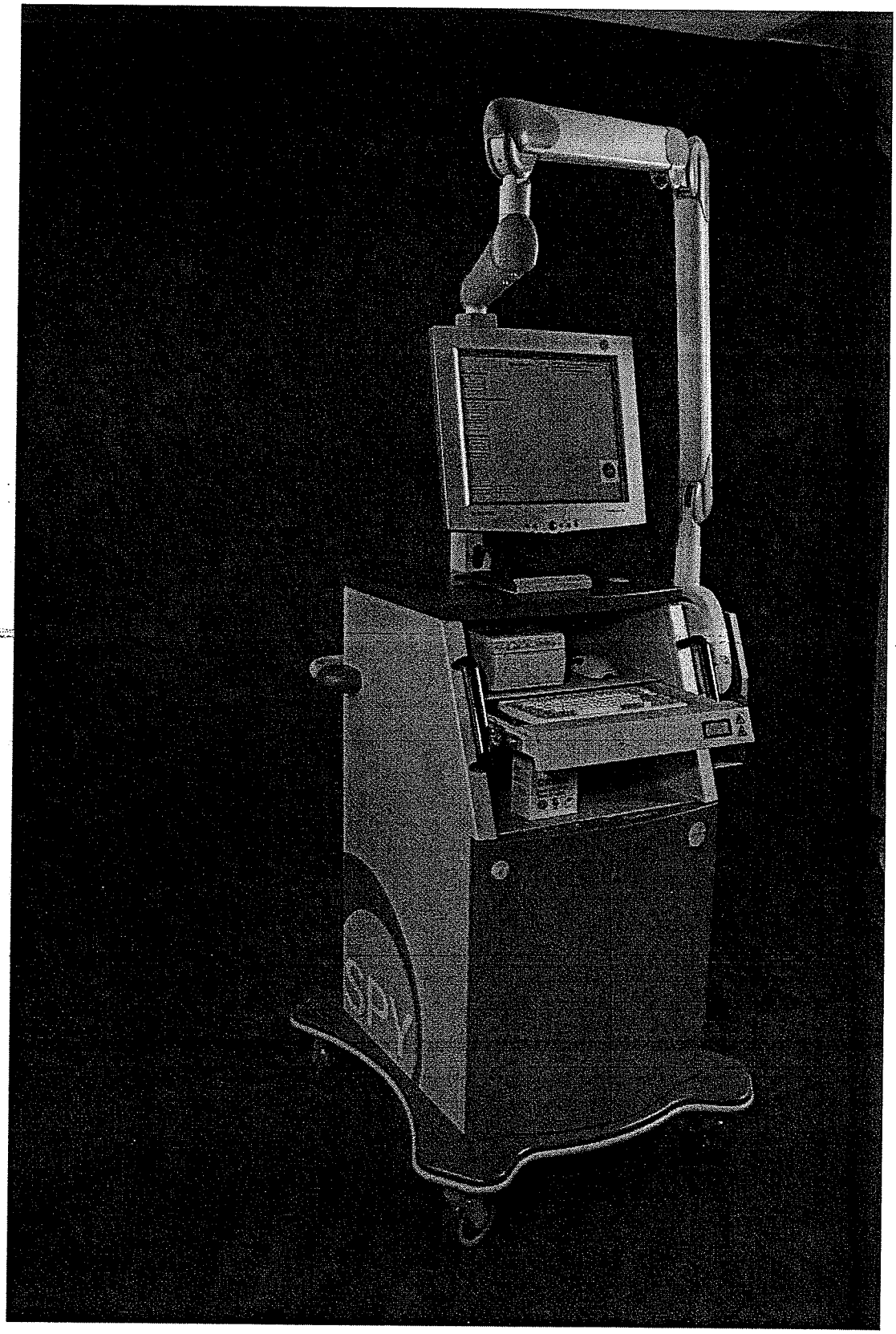


Figure VI: SPY 2000 System

For the purpose of this thesis, focus will be on the animal tests that were performed. The study and results are outlined below:

The effect of fluorescence image acquisition on the myocardium

Background

Numerous studies have demonstrated that ischemia reperfusion injury can lead to the generation of a variety of arrhythmias and it is believed that the occurrence of these arrhythmias is due in large part to the production of free radicals during the reperfusion of the ischemic myocardium (36,37,38,39). This notion has been supported by a number of studies, notably some elegant studies by Cole et al examining the electrophysiological effects of intra- (40) and extra- (41) cellular administration of free radical generating systems. There have been several reports citing that the excitation of ICG which results in fluorescence can lead to the generation of free radicals (42,43, 44, 45). These studies were primarily designed to assess the potential for ICG to be used as a photodynamic therapy agent (whose mechanism of action depends upon generation of free radicals) and generally concluded that ICG was a poor choice as a photodynamic therapy agent. Given the low laser power level and concentration of ICG used in the present application (35 mwats/cm^2 and 3μM) in conjunction with the limited time of exposure to laser light (35 seconds maximum) it was felt that there was a very limited possibility of generation of significant amounts of free radicals. However, there have been numerous instances of non cardiovascular drugs

causing QT prolongation leading to potentially fatal arrhythmias and it is essential to demonstrate the lack of such an effect in products coming to market (46).

Study Objectives

The present studies were designed to determine if the interaction of the laser light with ICG in the heart leads to clinically relevant arrhythmias or electrophysiological abnormalities that could lead to such arrhythmias. The studies were not designed to analyse the molecular basis or mechanism for arrhythmiagenesis and so it was felt appropriate to use analysis of the ECG as the best method for determining if electrophysiological abnormalities were occurring. As alluded to above, prolongation of the QT interval, which can lead to Torsade de Pointes which in turn can degenerate to ventricular fibrillation and sudden cardiac death, was of primary concern. Gross analysis of the ECG is an appropriate means to study the occurrence of clinically relevant arrhythmias. The studies were performed using a concentration of ICG 5 times higher than would be experienced under clinical conditions. In addition, the imaging protocol was repeated 10 times with minimal delay between each image sequence acquisition in order to test for potential cumulative effects (as might occur for example if 4 grafts were imaged in rapid succession upon completion of the surgical procedure).

The studies were also designed to determine if image acquisition had an effect on myocardial temperature. The laser, operating in the near infrared region of the spectrum, has the potential to increase myocardial temperature. It must be recognized however, that the low power density of the laser light on the

myocardium, <40 mwatts/cm^2, makes this a highly unlikely outcome. On the other hand, it could be argued that the presence of indocyanine green, which absorbs light at this wavelength, could increase the potential for this low intensity near infrared light to cause myocardial heating.

To determine if image acquisition caused cellular damage to the myocardium, circulating creatine kinase (CK) levels were determined prior to and upon completion of the imaging protocols. CK is an intracellular enzyme that is released into the circulation when the integrity of the cell membrane is compromised and is used as a marker of cell damage. Although CK is not specific for myocardial damage, any increase in circulating levels could be attributed to myocardial damage. The lack of increase in circulating CK between baseline and post imaging would be strong evidence for a lack of cellular damage to the myocardium during this period.

Materials and Methods

The study was performed at the National Research Council Institute for Biodiagnostics, Winnipeg, Manitoba, Canada. All procedures were approved by the Institute Animal Care Committee, consistent with Canadian Council on Animal Care (CCAC) guidelines.

Animal preparation

Six domestic pigs (male or female, mean body weight 78.3 kg, range 60.4 to 90 kg) were fasted overnight on the night prior to the study. Animals were premedicated with atropine (0.5 mg/kg), midazolam (0.4 mg/kg) and ketamine (20 mg/kg). Isoflurane in 30-35% O_2 (by mixing medical air and pure oxygen), 3%

initially, was given by mask and maintained at 1.5-3% following endotracheal intubation. The depth of anesthesia was controlled at the level at which the animal did not show spontaneous breath or muscular response to surgical procedures with a mean blood pressure of 60-80 mmHg by adjusting the level of isoflurane. The right carotid artery was cannulated for arterial pressure monitoring (using a Cobe 01-0415705OH transducer connected to a Gould TA 5000 Polygraph) and blood sampling. An intravenous line was established via the right jugular vein for venous administration of dye. The bladder was cannulated for urine collection throughout the experimental protocol. A sternotomy was performed and the pericardium opened longitudinally along the midline. A thermocouple (Physiotemp, model number 201-779-5577) was inserted 1-2 mm into the myocardium within the area of laser illumination during image acquisition and attached to a Cole Palmer Model 08500-40 temperature recorder.

Four ECG electrodes were attached to the skin as follows; RA 10 cm from midline at the level of the upper extremities, LA 10 cm from midline at level of upper extremities, LG between 1st and 2nd nipples and ground varied according to signal quality. Data were collected using a Grass Polygraph (Model number 79E), incorporating an ECG signal processing module (Low level preamplifier model 7PIJ and Amplifier model number 7DAH). Data were acquired using the Lead II setting. ECG data were collected for a period of 30 minutes in order to establish baseline parameters, throughout each of 10 consecutive image acquisition sessions and for 30 minutes upon completion of the 10th image sequence acquisition.

At the end of the studies, animals were euthanized by cardiac arrest under general anesthesia by means of a KCl bolus injected directly into the heart.

Creatine Kinase Determination

Plasma levels of creatine kinase (CK) were determined using the "CK NAC-activated creatine kinase kit" from Sigma Diagnostics. In this kit CK catalyses the reaction between creatine phosphate and ADP forming ATP and creatine. The ATP formed is utilized to phosphorylate glucose producingG-6-P in the reaction catalyzed by hexokinase. Subsequently, G-6-P is oxidized to 6-phosphogluconate (6-PG) in the presence of NADP. This reaction is catalyzed by glucose-6-phosphate dehydrogenase (G-6-PDH). During this oxidation, an equimolar amount of NADP is reduced to NADPH resulting in an increase in absorbance at 340 nm. The rate of change in absorbance is directly proportional to CK activity.

Blood samples were obtained immediately before starting the first image acquisition i.e. upon completion of the acquisition of 30 minutes of baseline ECG data and also upon completion of the 30 minutes post imaging ECG data acquisition. Duplicate samples of 0.1 ml plasma were analysed for each determination.

Image acquisition

The SPY™ 2000 device was positioned 30 cm over the exposed heart. The laser was activated simultaneously with injection of a 1 ml bolus of ICG (12.5 mg/ml) which was immediately followed by a flush of 5 ml saline solution. Each imaging

session lasted for 35 s. The imaging protocol was repeated a total of 10 times with a 1.5 minute interval between each image sequence acquisition.

Data Analysis

ECG traces were visually inspected for the occurrence of changes in the form of the cardiogram and for the occurrence of abnormal beats. The PR, QRS and QT intervals were measured manually. For each data point, intervals were measured on 5 cardiac cycles and averaged. Intervals were measured during each individual image sequence acquisition and every 2 minutes during both the 30 minute baseline and 30 minute post imaging ECG recordings. Arterial pressures were measured immediately before each imaging session and also during the last 10 seconds of each imaging session. Pressure data were acquired from 4 pigs.

During each imaging session, myocardial temperature measurements were recorded every 5 s.

Creatine Kinase levels were determined in duplicate and pre and post imaging values compared.

Results

Myocardial Temperature

The results of myocardial temperature measurements are presented in Table 1 (Appendix C) and in Figure VII. The results demonstrate that in all animals there was no significant change in temperature. .

In all animals only minor changes in temperature, were observed. The largest observed increase in temperature was 0.27°C (pig 4). In all animals the core body temperature was within the range 38.0-38.5°C. Myocardial temperature readings less than this likely reflect very superficial placement of the thermocouple within the myocardium. It is noteworthy that the largest observed increases in temperature occurred in those animals with the lowest baseline myocardial temperature and presumed superficial placement of the thermocouple. The outermost layer of tissue, potentially most at risk, is not exposed to temperatures outside of the physiological range during the imaging procedure. In some pigs the temperature decreased slightly over the first 10 – 15 seconds likely as a result of arrival of the 5 ml saline flush (at room temperature as is done in the clinical situation) into the coronary vessels.

Electrophysiological Parameters

There were no changes in the cardiograms of any of the pigs during each of the 10 imaging sequences and the 30 minutes immediately following the final imaging session.

The values for PR, QRS and QT intervals are presented in Tables 2, 3 and 4 (Appendix C) respectively. None of the measured intervals changed during image acquisition or during the 30 minutes immediately following the 10^{th} image sequence acquisition.

The results are presented in Figure VIIIa, VIIIb & VIIIc and illustrate no significant change in the intervals when comparing baseline to imaging and post-imaging.

Arterial Pressures

Systolic and diastolic pressures, measured in the carotid artery of 4 pigs, are presented in Table 5 (Appendix C) and illustrated in Figure IX. Administration of ICG and image acquisition had no acute significant effect on arterial pressures. Furthermore, over the course of 10 image acquisitions there was no decrease in arterial pressures indicating an absence of sub acute and also cumulative effects.

CK Release

All CK levels were within the normal range of 219 – 1411 Units/L for pig blood. Image acquisition did not cause an increase in circulating CK levels (Table 6: Appendix C) CK is an intracellular enzyme which should normally be present in the circulation at very low levels. The occurrence of elevated levels of circulating CK is indicative of cell damage such that the cell membrane has been compromised to an extent that the macromolecular CK can leak out. In this case CK can be taken as an index of myocardial cell damage. In recent years measurement of troponin isozymes has become the gold standard for assesing myocardial damage (47). However, measurement of CK can give valuable information relating to cellular damage. In this case we chose to use CK measurement as an index of myocardial cell damage because of the availability of a simple, reliable, cost effective and well described assay for this enzyme with no concerns related to lack of inter species cross reactivity of reagents as may have been the case with measuring troponins. While recognising that CK is not specific for myocardial damage we were not examining absolute levels of CK but rather we looked for increases in circulating levels of CK at times when the only

intervention being performed was the imaging protocol. The absence of an increase in circulating CK is strong evidence that the imaging procedure did not cause myocardial cell damage.

No clinical or physiologically statistically significant differences: Conclusions

1. Image acquisition using the SPY™ 2000 device does not lead to statistically significant physiological increases in myocardial temperature.
2. The interaction of laser light and ICG within the coronary vessels does not cause statistically significant electrophysiological abnormalities.
3. Administration of ICG, at doses 5 times higher than would be experienced in clinical situations, does not affect arterial pressures.
4. The interaction of laser light and ICG, at 5 fold higher concentrations than would be experienced clinically, does not affect arterial pressures.
5. Image acquisition did not cause cellular damage to the myocardium as determined by a lack of increase in Creatine Kinase release following image acquisition.

Figure VII: Myocardial Temperature During Fluorescence Image Acquisition

Myocardial temperature was measured in six pigs by means of a thermocouple inserted 1-2mm deep in the epicardial surface. Ten imaging sequences were collected with a 1.5 min. interval between each image acquisition. B = temperature measured at baseline between image sequences i.e. temperature measured during image acquisition.

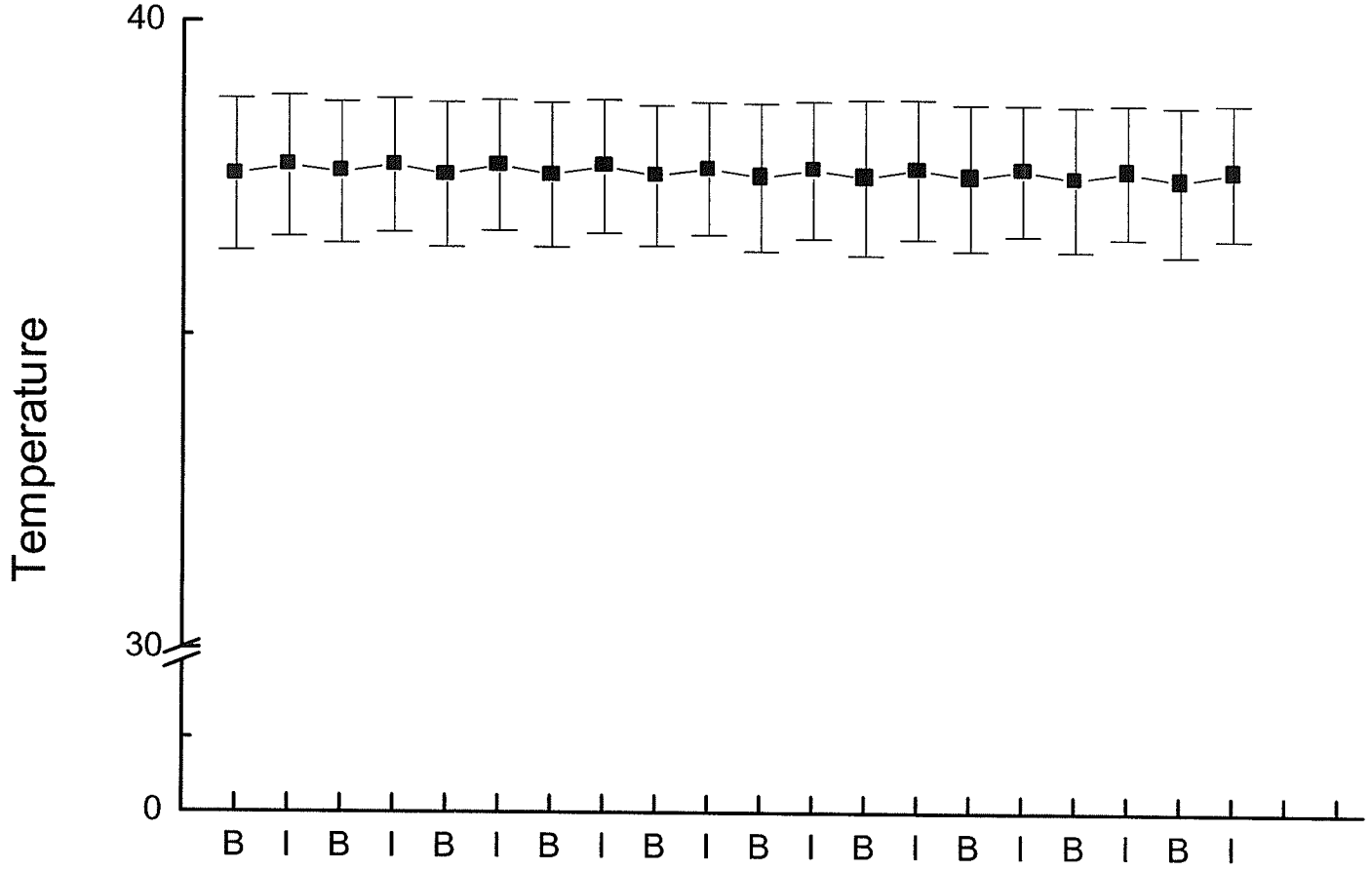


Figure VIIIa: Effect of Fluorescence Imaging on QT interval

Electrophysiological parameters were measured 30 min. prior to imaging during each of 10 image acquisition sequences and for 30 min. post imaging. B1-B15 = baseline measurements taken at 2 min. intervals. I1-I10 = data acquired during each of 10 consecutive image sequences. P1-P15 = data acquired during 15 consecutive 2 min. intervals following acquisition of all imaging sequences.

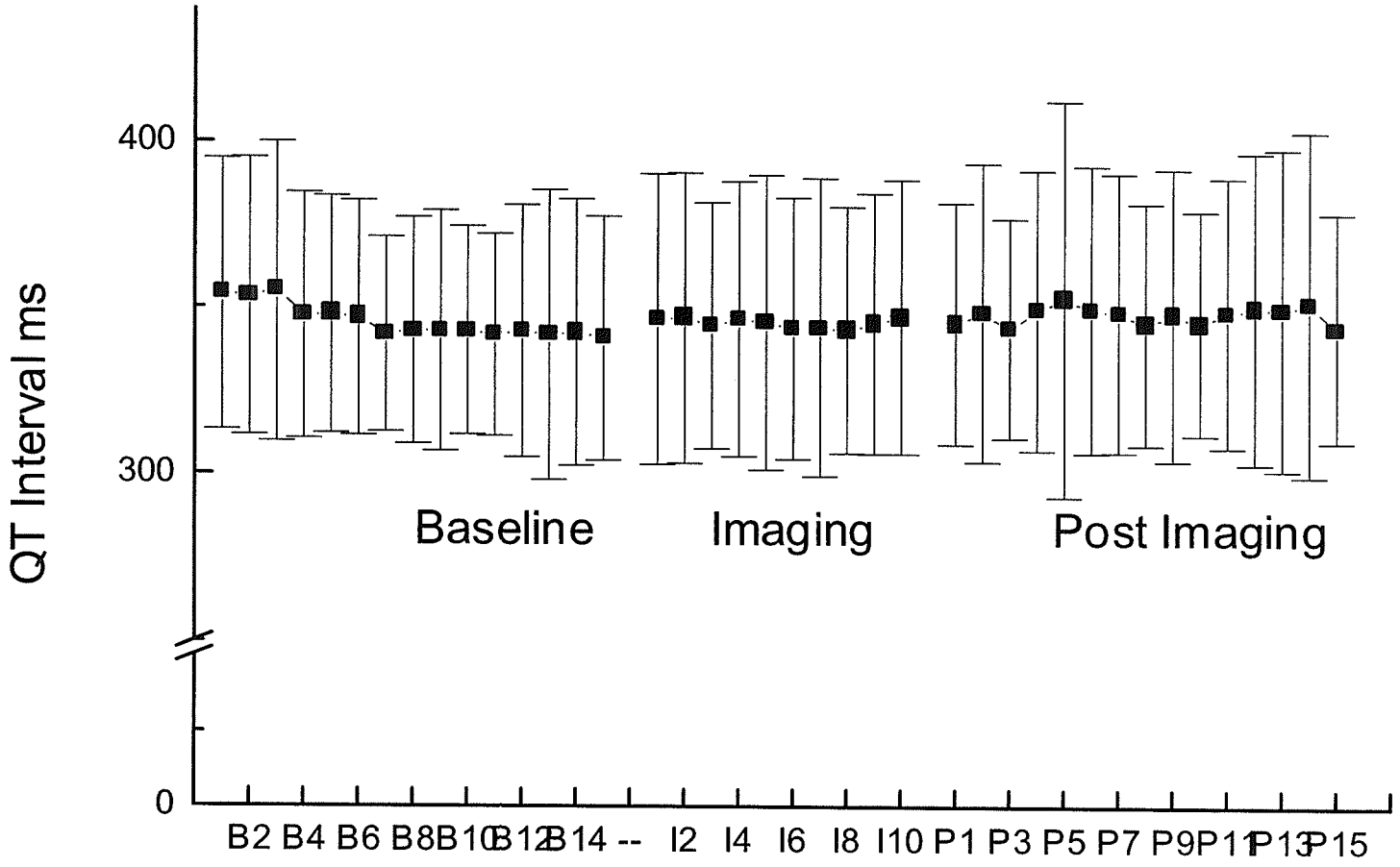


Figure VIIIb: Effect of Fluorescence Imaging on QRS interval

Electrophysiological parameters were measured 30 min. prior to imaging during each of 10 image acquisition sequences and for 30 min. post imaging. B1-B15 = baseline measurements taken at 2 min. intervals. I1-I10 = data acquired during each of 10 consecutive image sequences.
P1-P15 = data acquired during 15 consecutive 2 min. intervals following acquisition of all imaging sequences.

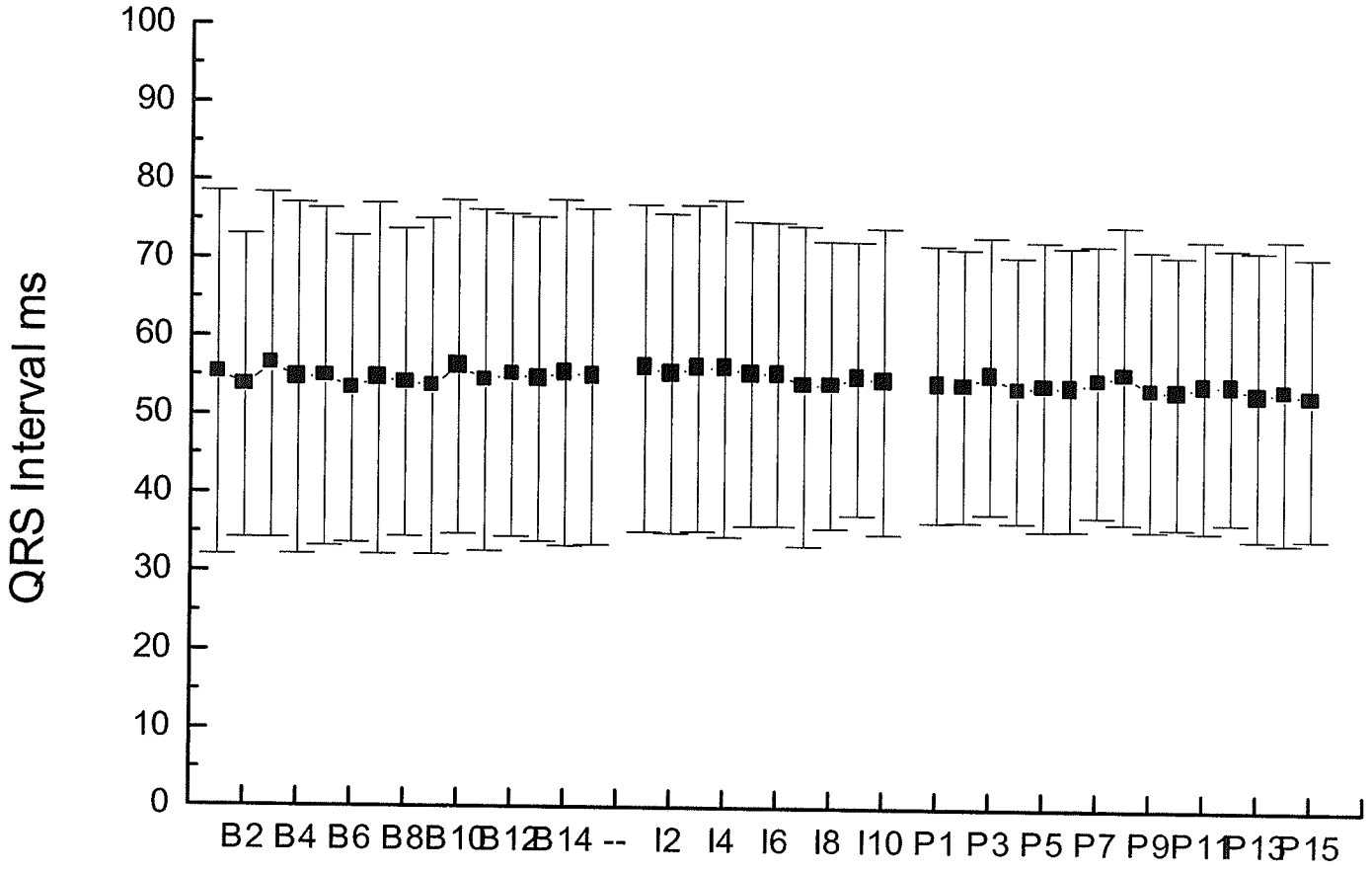


Figure VIIIc: Effect of Fluorescence Imaging on PR interval

Electrophysiological parameters were measured 30 min. prior to imaging during each of 10 image acquisition sequences and for 30 min. post imaging. B1-B15 = baseline measurements taken at 2 min. intervals. I1-I10 = data acquired during each of 10 consecutive image sequences.
P1-P15 = data acquired during 15 consecutive 2 min. intervals following acquisition of all imaging sequences.

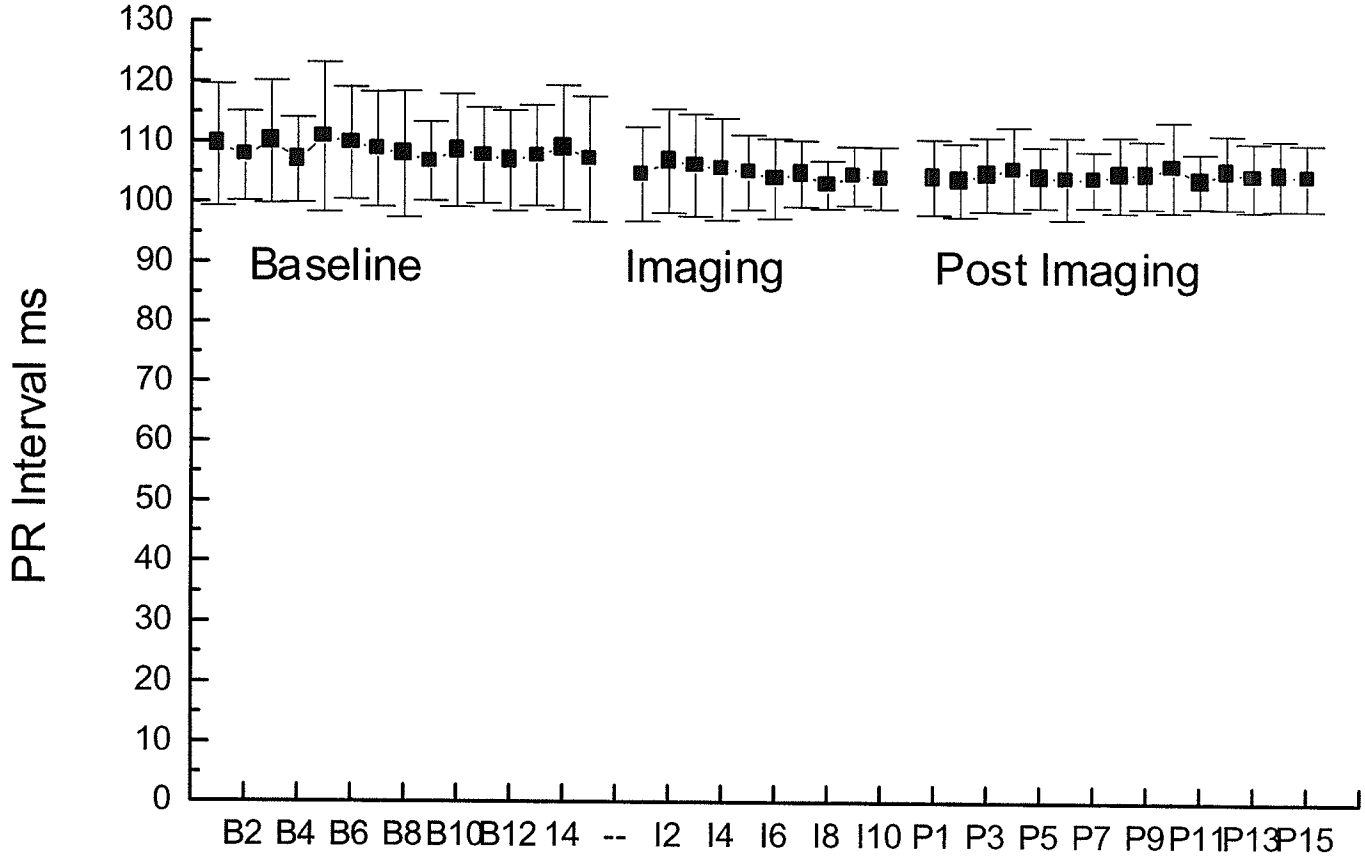
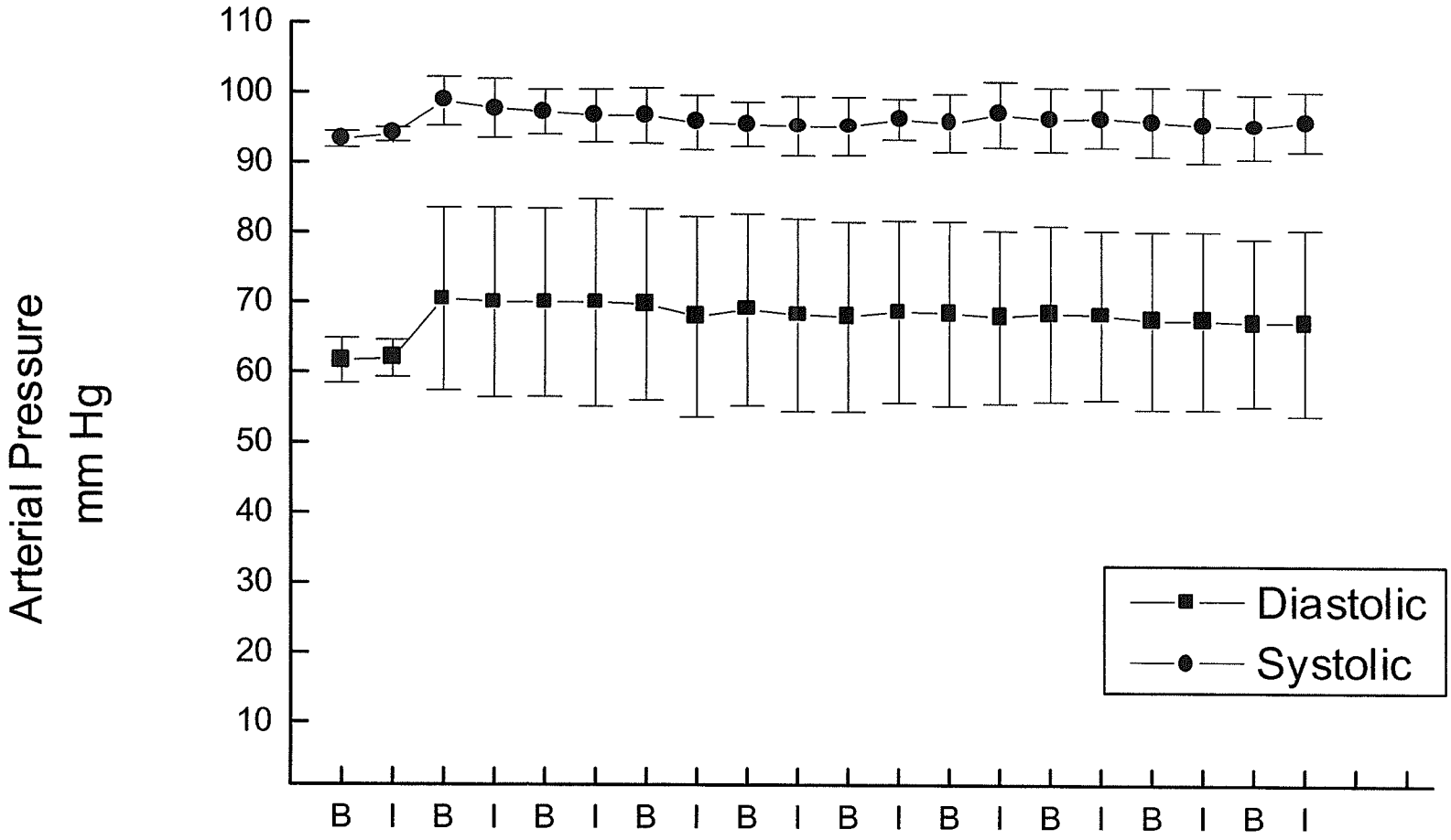


Figure IX: Effect of Fluorescence Imaging on arterial pressure

Arterial pressures were measured in four pigs (n=3 for first two data points). Note: increase between 2nd and 3rd data points is because the pig for which no data were available for data points 1 and 2 had higher systolic and diastolic pressures than the other pigs. B = pressures recorded at baseline. I = pressures recorded during image acquisition.



CHAPTER IV

DRUG PHARMACOLOGY & TOXICOLOGY

Indocyanine Green Dye Overview

Indocyanine green has been approved for use in humans and has been safely used for over 40 years for measurement of cardiac output, assessment of hepatic function and for ophthalmic angiography. The incidence of adverse reactions to the clinical use of indocyanine green is low and adverse reactions are summarized below. In general, most reactions to ICG are rare and mild (sore throat, feeling of warmth) although a few of the reported adverse effects have included hypotension requiring treatment with epinephrine.

The first four reports of adverse reactions received by the manufacturer of ICG occurred over a time interval during which 240,000 doses were sold (31) A literature review published 34 years following the introduction of ICG compiled only 17 reported reactions including 2 deaths. However, both deaths occurred in patients undergoing cardiac catheterization and it was concluded that it was complications arising from the catheterization procedures that resulted in death. Seven of the 17 reactions were in hemodialysis patients, suggesting a contraindication in uremic patients undergoing hemodialysis (32). One additional death has been reported in Japan (33). Three additional case reports were reported in a literature review that concluded that there were no data linking reactions to pre-existing allergies (34).

A prospective study involving 1,923 consecutive ICG video-angiograms in 1,226 patients encountered 8 reactions of varying intensity, one of which was judged to be severe (35).

Indocyanine green dye (ICG) was chosen for this application after an extensive search through the literature. In order to allow for the commercial success of an imaging system it was recognized that an agent with the following characteristics would be required: readily available, safety history, regulatory approval for clinical use, ability to fluoresce in the near-infrared (NIR) region of the spectrum. The rationale for these characteristics are outlined below:

(i) Readily available: in order to be used in a procedure that is performed approximately 1 million times annually an agent was required that could be readily manufactured and distributed worldwide.

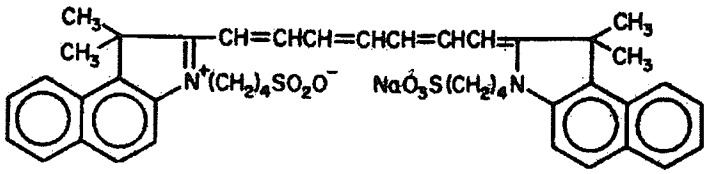
(ii) Safety history: bypass surgery is a relatively complex procedure performed under the influence of anesthesia. Furthermore, the patients are normally using a multitude of other drugs (blood pressure, cholesterol lowering etc) and as such the agent used for imaging must have a strong safety profile that has been demonstrated in varying populations. The surgeons must feel that they are using a compound they know and trust; based on its reported use in the literature and in their own hospitals.

(iii) Regulatory approval: ICG is approved in the US, Europe and Asia for the following applications; ophthalmic angiography, cardiac output and liver function tests. These approvals will allow for an easier passage through the regulatory processes although the drug will be used for a new indication.

(iv) Near infrared (NIR) sensitivity: it was recognized that the drug should work in the NIR region of the spectrum in order that tissues could be penetrated upto 3mm (required for vascular imaging). Although other agents were available that could allow for further penetration they worked in regions of the spectrum (e.g. X-ray) that would require safety equipment for the operators which was unacceptable.

Chemistry

Indocyanine green is a sterile, water soluble tricarbocyanine dye with a molecular weight of 775. The empirical formula is C43H47N2NaO6S2. The dye in its final form contains sodium iodide (<5%). ICG has a peak spectral absorption in the 800-810nm range in plasma or blood with an emission peak of 835nm. The chemical structure for ICG is given below:



Pharmacokinetics

Absorption

Indocyanine green is administered by intravenous injection and thus has a bioavailability of 1. Fluorescence images of a vascular bed are acquired during the first pass of the agent through the field of view and so there are no concerns relating to the first pass extraction of ICG by the liver.

Distribution

Indocyanine green is extensively bound to plasma proteins and is confined to the vascular space. Cherrick et al (48) were the first to demonstrate this in 1960 in a study in which they compared the initial volume of distribution of ICG with the volume of distribution of I^{121} labeled albumin. Four normal subjects were given approximately 20 μCi of the labeled albumin and 50 mg of ICG in a rapid intravenous injection and venous samples were taken for analysis at intervals over the following 12 minutes. The distribution volume for ICG, 3.48 ± 0.72 litres, was not significantly different from the distribution volume for albumin (3.46 ± 0.58). Using starch gel electrophoresis, Cherrick et al (48) concluded that the ICG was bound to albumin (95%) with only minor binding to other plasma proteins. The value for the volume of distribution has been confirmed in a study by O'Reilly (49), comparing spectrophotometric and high performance liquid chromatography (HPLC) techniques for estimating the clearance of ICG in a group of 5 patients with chronic liver disease (49). The calculated volumes of distribution, 3.23 ± 1.15 and 4.95 ± 2.71 for spectrophotometric and HPLC determinations respectively, were not significantly different and confirm that ICG is confined to the intra-vascular space.

Thirteen studies utilizing sensitive analytical techniques (gel permeation chromatography on Sephadex G-200 or differential ultra- centifugation) have investigated the identity of the plasma protein responsible for ICG binding in human, dog and pig plasma (48). These studies revealed that ICG binds to a variety of lipoproteins with HDL_3 being the major carrier protein for the dye in all

species studied, with binding to albumin representing a minor component of ICG binding. In man, HDL_3 accounts for approximately 50% of the total binding of ICG, with albumin accounting for 2-30 % of the total binding capacity and the remainder associated with VLDL, LDL and HDL_2 (48)

Metabolism

Indocyanine green does not undergo metabolism and is excreted unchanged by the liver. Recent reports have discussed the presence of an ICG "degradation product" (48). This "degradation product" is not formed in vivo but rather is formed upon dissolution of the ICG in aqueous solution. Mass spectroscopy studies suggest that the degradation product is, in fact, a dimer formed from two ICG molecules (48).

Elimination

Indocyanine green is eliminated by the liver with no metabolism or conjugation and does not undergo enterohepatic recirculation. Clearance of ICG from the blood of normal subjects was described as "occurred in nearly exponential fashion for 10 to 20 minutes. Following that initial period, deceleration of decay occurred in all cases" (48). The half life in normal individuals was 3.4 ± 0.7 minutes while for patients with liver cirrhosis, the half life was 25 minutes (range 3.3 to 110 minutes). There was no trace of ICG in the urine collected from 5 patients over the initial 6 hours following injection of 2 mg/kg ICG. In two patients (having recently undergone cholecystectomy for gallstones) ICG was detected in

bile obtained from T-tube drainage at 8 and 15 minutes following injection of ICG (22). The peak concentration in bile occurred at 120 minutes following injection and was present in trace amount at 19 hours post injection. The plasma half life of < 5 min has subsequently been confirmed in numerous reports

Paumgartner (24) undertook an intensive study of the removal of ICG from Sprague Dawley rats by injecting ICG into the left jugular vein and sampling blood through a right carotid artery cannula at 0, 2, 3, 4, 5, 6 , 9 and 12 minutes following ICG injection. The initial rate of removal was determined by means of spectrophotometric determination of ICG concentration in the blood samples. The initial rate of removal increased in a non linear manner with increasing dose. Plotting the data in a Lineweaver-Burke plot demonstrated that the relationship between ICG removal rate and dose followed Michaelis-Menten kinetics (24). From these data, a maximum initial ICG removal rate (V_{max}) of 0.38 (confidence limits 0.34 – 0.43) μmoles/min.100g body weight and an apparent Michaelis constant (K_m) of 0.5 μmoles/100g body weight were determined for normal rats. Similar analysis of ICG removal in normal humans gave values of 5.39 (CI 4.04 – 7.17) μmoles/min.kg and 18.18 (12.43 – 25.72) μmoles/kg for V_{max} and K_m respectively. For fatty livers the values were 2.18 and 8.06 for V_{max} and K_m respectively while for cirrhotic livers the values were 1.02 and 5.51. These parameters for a saturable elimination pathway have implications primarily for studies making use of ICG to study hepatic function where greatest sensitivity of the test will be achieved by selection of an appropriate (i.e. saturating) test dose.

It is evident that saturating doses may only be achieved in cases of severe liver disease.

After uptake by the liver, the ICG is secreted into the bile in high concentrations (48). Recovery of ICG in bile following intravenous injection accounted for 90 – 100 % of the injected dose in rats, dogs and rhesus monkeys. It may be concluded that ICG is rapidly taken up into the hepatocyte and then, after a delay, gradually secreted into the biliary tree.

Toxicology

Indocyanine green is generally well tolerated and displays a wide margin of safety. Sixty four mg/kg has been administered to rats and 25 mg/kg to rabbits (50) with no reported mortality. The LD_{50} for indocyanine green in mice is 60 mg/kg (50). The acute toxicity was studied in 7 male Swiss-Webster mice following intravenous administration of 35 mg/kg of indocyanine green in 0.1 ml (50). Two mice were sacrificed 2 days following administration of the ICG and the remaining 5 were sacrificed on day 14. The inguinal, caudal and lumbar lymph nodes were removed first followed by the spleen, liver and kidneys. The tissues were formalin fixed, sectioned and stained with Ehrich's hematoxylin and eosin. No histological abnormalities were noted in any of the sections.

Bile flow was inhibited in rats during constant infusion of ICG at rates of 0.33, 0.66 and 1.33 mg/kg/min (50). At 90 min of infusion at a rate of 0.66 mg/kg/min the bile flow was 20% of normal while 90 min infusion at 1.33 mg/kg/min caused a complete cessation of bile flow. Less dramatic effects were observed in rabbits and dogs. In the dog a constant infusion of 0.532 mg/kg/min caused an

approximate 40% decrease in bile flow. In these studies a single bolus injection of ICG caused decreased bile flow in rats and rabbits at doses of 32 mg/kg or higher. In the dog rapid injection of 1 or 4 mg/kg had no effect on bile flow. It was demonstrated in male Wistar rats that ICG inhibited bile flow while bile salt output exhibited only minor changes, suggestive of interference with a bile-salt independent fraction of bile (50)

CHAPTER V

CLINICAL DATA

The SPY™ system has been utilized in assessing 3600 artery and vein grafts during off pump (without cardiopulmonary bypass) and on pump (with cardiopulmonary bypass) CABG surgery in humans in Europe, Japan and Canada. Published or in-press studies are presented herein (see Table I). The system was used in routine clinical practice as the device has CE Mark (Europe), Shonin (Japan) approval as well as approval in Canada. The literature reports that the SPY™ system was able to non-invasively, quickly and safely identify 17 conduits in 311 patients that required revision during the surgical procedures (51, 52, 53 54, 55). In all cases, the lack of patency was visualized clearly by the SPY™ system using doses of ICG well below that approved for human use, allowing the surgeon to revise the graft thus decreasing subsequent myocardial infarctions and the morbidity and mortality associated with poor graft patency. There were no reported adverse effects of using the SPY™ system with the ICG as demonstrated by monitoring of cardiac, renal and hepatic function.

Human Experience

Takahashi et al (51) reported use of the SPY™ system in 72 patients in Japan. Two hundred and ninety grafts were assessed with 11% of patients being operated on off-pump and 89% on-pump. A bolus of ICG (2.5 mg in 1.0 ml) was administered and the SPY™ system used to visualize the grafts. Four (1.4%) grafts were clearly determined to lack patency and were revised during the surgical procedure. Two of the grafts failed because of defective proximal anastamoses and a further two because of defective distal anastamoses.

Following surgical revision after SPY™ use all grafts demonstrated excellent flow. The authors conclude that the SPY™ system offers the great advantages of non-invasive, safe assessment of graft patency with no adverse effects.

Reuthebuch et al. (52) reported a case study in the Annals of Thoracic Surgery in 2003 based on their experience in one patient with three grafts in Zurich, Switzerland. Following one graft procedure with a sutureless anastamotic device it was determined that there was an occlusion of the proximal graft due to mobile atheroma plaque. The SPY™ system used with a 0.5 ml intravenous injection of ICG clearly, quickly and safely identified the occlusion and the graft was revised during the surgical procedure. Postoperative EKG showed no ischemic alterations nor any elevated cardiac enzymes (as determined by CK, CK-MB, Troponin T) nor were there any elevated renal (serum creatinine, urea) or liver (ASAT, ALAT, LDH) enzymes. The authors conclude that the SPY™ system demonstrated immediate and more precise diagnosis of graft occlusion as compared to a flowmeter which is routinely used at their institution.

Taggart et al (53) reported their initial use of the SPY™ system in 84 patients with 213 conduits at John Radcliffe Hospital in Oxford. Balacumaraswami et al (54) expanded the report to include 533 grafts in 200 patients, including the initial report by Taggart et al. A bolus of ICG (1.25 to 2.5 mg; 2.5 to 5 mg in patients > 100 kg) was administered through the central venous line followed by imaging with the SPY™ system. In this group of 533 conduits it was determined by the SPY™ system that 8 (1.5%) lacked patency. Six (6.9%) of these grafts were done off pump and 2 (4.4%) were done on pump. In all cases use of the SPY™

system provided imaging of the coronary vasculature and revised grafts during the surgical procedure demonstrating excellent flow. There were no reported adverse effects of using the SPY™ system.

Electrocardiograms of 30 patients were evaluated by the Core Cardiovascular Analysis Laboratory at Stanford University (acquired prior to CABG and post-CABG surgery). No PR, QRS or QT prolongations, acute ischemia or infarction were observed, demonstrating no cardiac effects of using the SPY™ system with ICG. The authors conclude that the SPY™ system allows verification of graft patency and immediate correction of failed grafts and should be considered for routine use in CABG procedures.

Reuthebuch et al. (55) reported their experience with 38 patients using the SPY™ system to evaluate 124 grafts during off pump surgery. A bolus of 0.03 mg/kg body weight of ICG was administered and followed by imaging with the SPY™ system. Seventeen of these grafts could not be visualized with the system due to posterior location with overlapping sternum or pericardium. In the remaining 107 grafts, 4 (3.7%) were found to require revision. Three were due to anastamotic constriction and one due to graft dissection. All grafts demonstrated excellent flow following SPY™ use and graft revision. All patients were monitored for cardiac, renal and hepatic function 4 hours following surgery and every consecutive day until discharge. No acute or long-term effects of ICG use were observed as demonstrated by liver (ASAT, ALAT, LDH), renal (serum creatinine, urea), and cardiac (CK, CK-MB, Troponin T, EKG) function monitoring. The authors conclude that use of SPY™ imaging results in 100% on-

table graft patency and competes with radiographic angiography, the "gold standard" for assessment of graft patency.

Representative images as seen in the operating room using the SPY System are shown in Figures I – IV.

Table I: Human Experience Summary

Author	Takahashi et al (51)	Reuthebuch et al (52)	Taggart et al (53)	Balacumaraswami et al (54)	Reuthebuch et al (55)
Publication	ICVTS 2004;3:479-483	Ann Thorac Surg 2003;75:1626-9	Ann Thorac Surg 2003;75:870-3	J Thorac Cardiovasc Surg 2004: 128:238-44	Chest 2004;125(2):418-24
Patients	72	1	84	200 (including 84 from Taggart et al)	38
Grafts	290	3	213	533	107 of 124 [17 grafts could not be imaged due to posterior location (CX, RCA) with overlapping sternum or pericardium]
Mean Grafts Per Patient	4.0	3.0	2.5	2.7	3.3
Operation	11 % Off Pump 89 % On Pump		77 % Off Pump 23 % On Pump	78 % Off Pump 22 % On Pump	All Off Pump
Graft Locations	79 LAD 40 Diagonal 13 Intermediate 57 Obtuse Marginal 31 CX 36 Posterior Descending 15 Posterior Lateral 12 RCA 7 RV Branch	1 Left Main 1 LAD 1 RCA	77 LAD 52 Obtuse Marginal 17 Diagonal 42 PDA 24 Other		37 LAD 28 Diagonal 17 Obtuse Marginal 11 CX 31 RCA
Graft Failures	4 (1.4 %) 2 Proximal 2 Distal	1 (33 %)	4 (1.9 %)	8 (1.5 %)	4 (3.7 %) 3 Anastamotic Constriction 1 Graft Dissection
Graft Revisions	4	1	4	8 6 (3.9 %) Off Pump 2 (4.4 %) On Pump	4
Outcome	All demonstrated excellent flow following SPY™ use and graft revision.	Demonstrated excellent flow following SPY™ use and graft revision. No liver enzyme (ASAT, ALAT, LDH) or renal (serum creatinine, urea) elevations were observed. Imaging	All demonstrated excellent flow following SPY™ use and graft revision.	All demonstrated excellent flow following SPY™ use and graft revision. EKGs of 30 patients were submitted to Stanford University's Core Cardiovascular Lab and demonstrated no EKG changes.	All demonstrated excellent flow following SPY™ use and graft revision. No liver enzyme (ASAT, ALAT, LDH) or renal (serum creatinine, urea) elevations were observed. Imaging had no detrimental effects on the myocardium (CK, CK-MB,

		had no detrimental effects on the myocardium (CK, CK-MB, Troponin T, EKG).			Troponin T, EKG).
Conclusion	SPY™ offers great advantages of non-invasive, safe assessment of graft patency.	SPY™ demonstrated immediate and more precise diagnosis of occlusion of proximal vein graft due to mobile atheroma plaque as compared to flowmeter.	SPY™ demonstrated similar quality to conventional angiography.	SPY™ allows verification of graft patency and immediate correction of failed grafts and should be considered for routine use in CABG patients.	SPY™ imaging competes with radiographic angiography, the "gold standard" for assessment of graft patency. With this technique a 100% on-table patency rate seems possible.

LAD = Left Anterior Descending Artery
RCA = Right Coronary Artery
RV = Right Ventricular
PDA = Posterior Descending Artery
CX = Circumflex

Figure I: Intraoperative indocyanine green angiogram of a skeletonized left internal thoracic artery (white arrows) with sequential distal anastomoses (Calafiore, Italy).

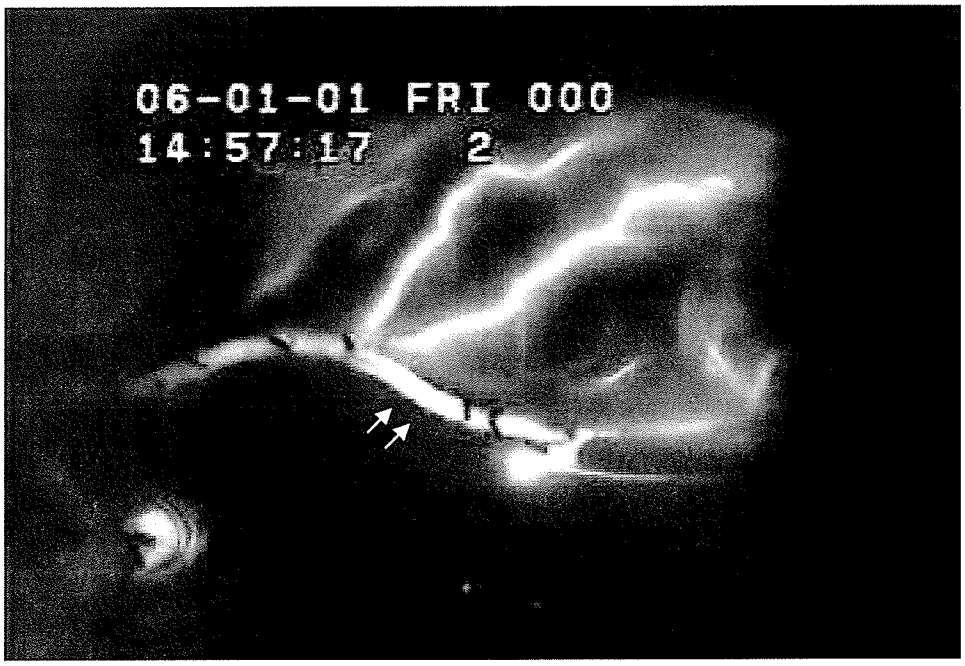


FIGURE II: RIMA to LAD
(Taggart, UK)

FIGURE III: LIMA to OM1
(Taggart, UK)

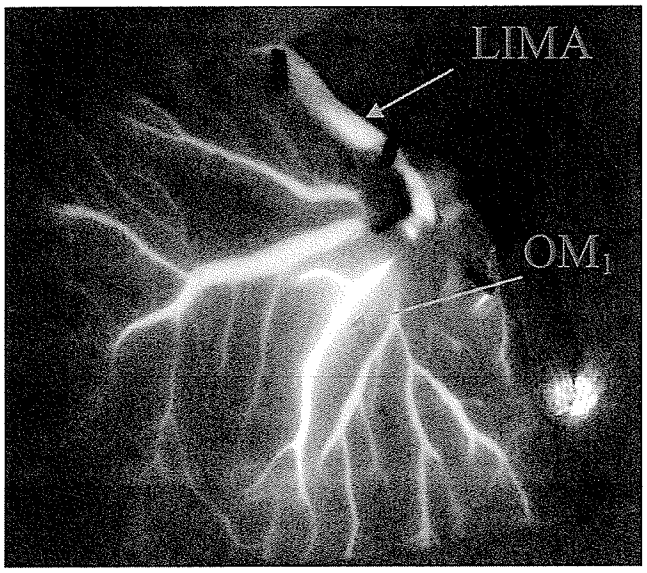


FIGURE IVa: Intraoperative indocyanine green angiogram showing no flow in a saphenous vein graft (white arrows) (Fremes, Canada).

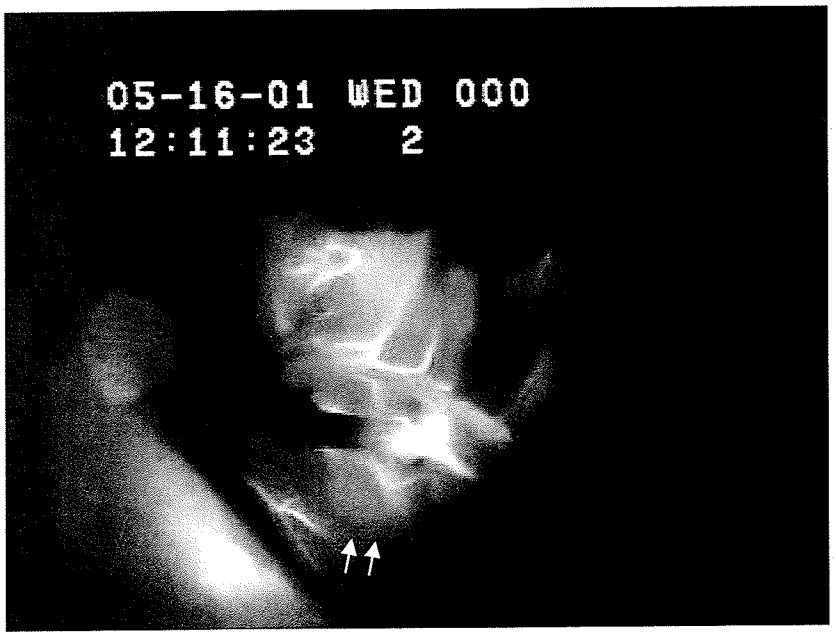
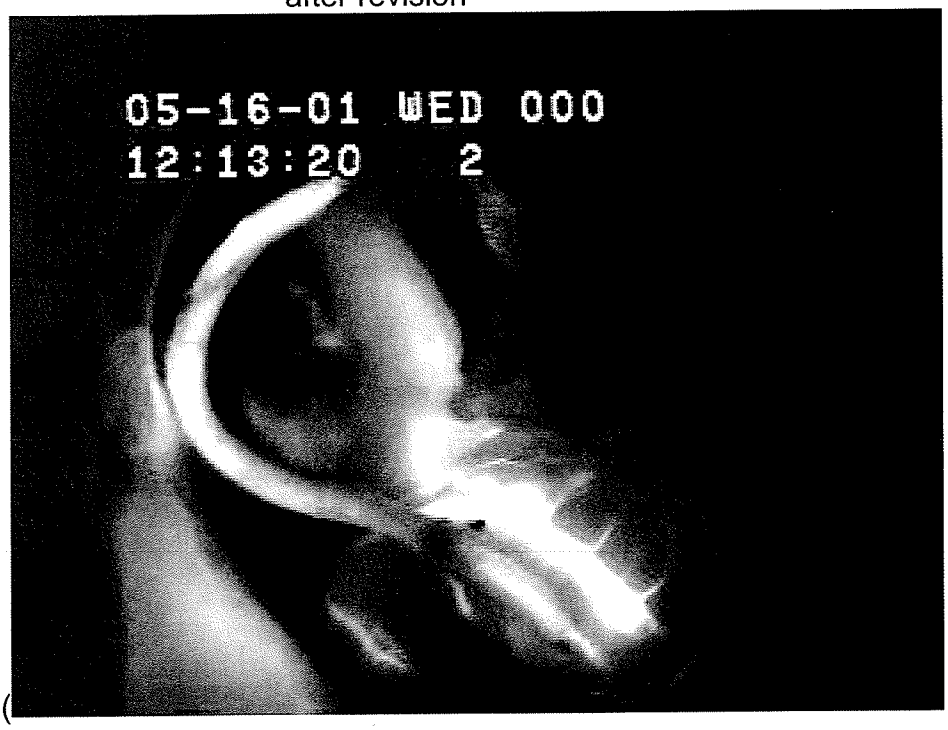


FIGURE IVb: Intraoperative indocyanine green angiogram showing flow into previously twisted saphenous vein graft (white arrows) after revision



(

CHAPTER VI

DEVICE DESCRIPTION

The SPY™ system enables cardiovascular surgeons to visualize the coronary vasculature and bypass grafts intraoperatively during CABG surgery. The system provides the surgeon with the capability to view, record and replay infrared fluorescent images of the coronary vasculature and grafts on the surface of the heart.

Indocyanine green (ICG) is administered intravenously through the central venous line, directly into the bypass pump or via the cardioplegia line. While ICG distributes through the coronary vasculature, a 806 nm light source illuminates a 7.6 X 7.6 cm area of the heart surface. Absorption of the laser light causes excitation of the ICG followed by emission of infrared energy at a wavelength of 830 nm. This results in a fluorescent image of the coronary blood vessels and bypass grafts. A CCD camera with an 805 nm cut-on optical filter captures the images. Up to 34 seconds of images are captured within one sequence and are monitored on the display and recorded by a computer for storage on a CD. Once the image is captured the laser automatically shuts off. The images are used to evaluate the integrity of the coronary vessels and bypass grafts during the CABG procedure.

The product's electrical and optical characteristics are configured as shown in Figures I and II. These figures include a schematic of the device and components.

The laser illumination lens, optical filters, the CCD camera, and focus detector are contained in an imaging head. It is mounted on an articulating arm that is

positioned 30 cm above the heart. A mouse control is used for initiating the laser and for replaying the images. The articulating arm stays in place once positioned, and contains the electrical cables to the camera and the fiber optic bundle from the laser. A sterile drape covers the imaging head and articulating arm. The arm is mounted on a mobile cart with locking wheels allowing the stand to be positioned in place in the operating room. A high resolution LCD is mounted on the cart and can be positioned for optimal viewing. The main power switch, isolation transformer, laser, computer with CD recorder, printer, camera power supply and laser power supply are on the base of the cart.

A collimated laser beam is produced by the laser diode and is conducted to the imaging head through the laser delivery fiber. The laser is decollimated and made incoherent by the illumination lens which spreads out the light to a 7.6 X 7.6 cm area on the heart surface. The emission filter allows for transmission of infrared radiationat wavelengths greater that 815 nm, centered at 830 nm. The CCD camera captures the image and relays it to the monitor for display, to the computer for storage on a CD and to the printer.

Figure III shows a current version of the device (Spy™ 2000) in use.

Procedure

Once the CABG procedure(s) is completed, the imaging head is positioned over the heart. Initial focus is achieved by observing a depth gauge showing 30 cm distance from the camera to the heart surface. Focus is confirmed by looking at a picture of the heart on the monitor. The laser and CD recorder are activated and the ICG is injected through the central venous line. The monitor is observed

in real time for passage of the ICG through the coronary vasculature and bypass graft. It takes approximately 30 seconds for the ICG to pass through the coronary vasculature and graft. The laser is automatically shut off after 34 seconds of illumination. During imaging, the ICG is visualized sequentially filling various vessels of the coronary beds (coronary arteries, microvasculature and veins). There is no software processing of the imaging involved in this procedure. The Operators Manual for full system operation is included in Apeendix A.

Laser Specifications:

Type	Coherent Semiconductor Group F-81-2600C-200B
Classification	IIIb
Power Input	5 VDC
Power Output (post decollimated)	2 W
Laser Beam	Decollimated and made incoherent
Field of Illumination	7.6 X 7.6 cm
Distance to Object	30 cm
Angle of Dispersion	246 mrad
Power Density at the Heart	40 mW/cm^2 (ANSI standard for MPE to skin is 327 mW/cm^2)
Radiant Power	35 mW through 7 mm aperture at 20 cm
Time of Laser Illumination	34 sec
Laser Power Supply	5 VDC/6 amps
Laser Driver	Wavelength Electronics PLD-5000

Camera Specifications:

Type	Hitachi KPM2RN
Image	Black and White
Horizontal Resolution	570 TV lines
Minimum Illumination	0.3 lux at f/1.4
S/N	56 db
Lens	Rodenstock Precision Optics 25 mm f/1.4
Working Aperture	f/8
Power Supply	Astec ACV 103
Emission Filter	Barr optics custom made 815 nm cut-on filter

Mechanical Specifications:

Articulating Arm	Gendex 765 DC
Arm Reach	168.9 cm
Arm Range	54 - 168.9 cm
Degree of Freedom	6
Monitor	Samsung 170T or Samsung 172B
Printer	Sony UP-895MD
Computer for Image Storage	Pentium IV with flat panel monitor, keyboard and optical IntelliMouse

Other System Components:

Isolation Transformer	Toroid 908.1202
Distance Sensor	SICK Optics WTA24
Cart	MPE custom design
Control Box Enclosure	Custom design
Condenser Lens	Melles Griot, 01 CMP 109, air-spaced pyrex condenser lens, 25 mm focal length
Fiber Patch Cord	Thorlabs M17L05, 5 meter 0.22 NA 200 micron core with SMA connectors
Light Guide	CMED/RELA custom design
Sterile Drape	Microtek Medical, Inc. #2908 universal laser/microscope drape

Figure I: Electrical Configuration

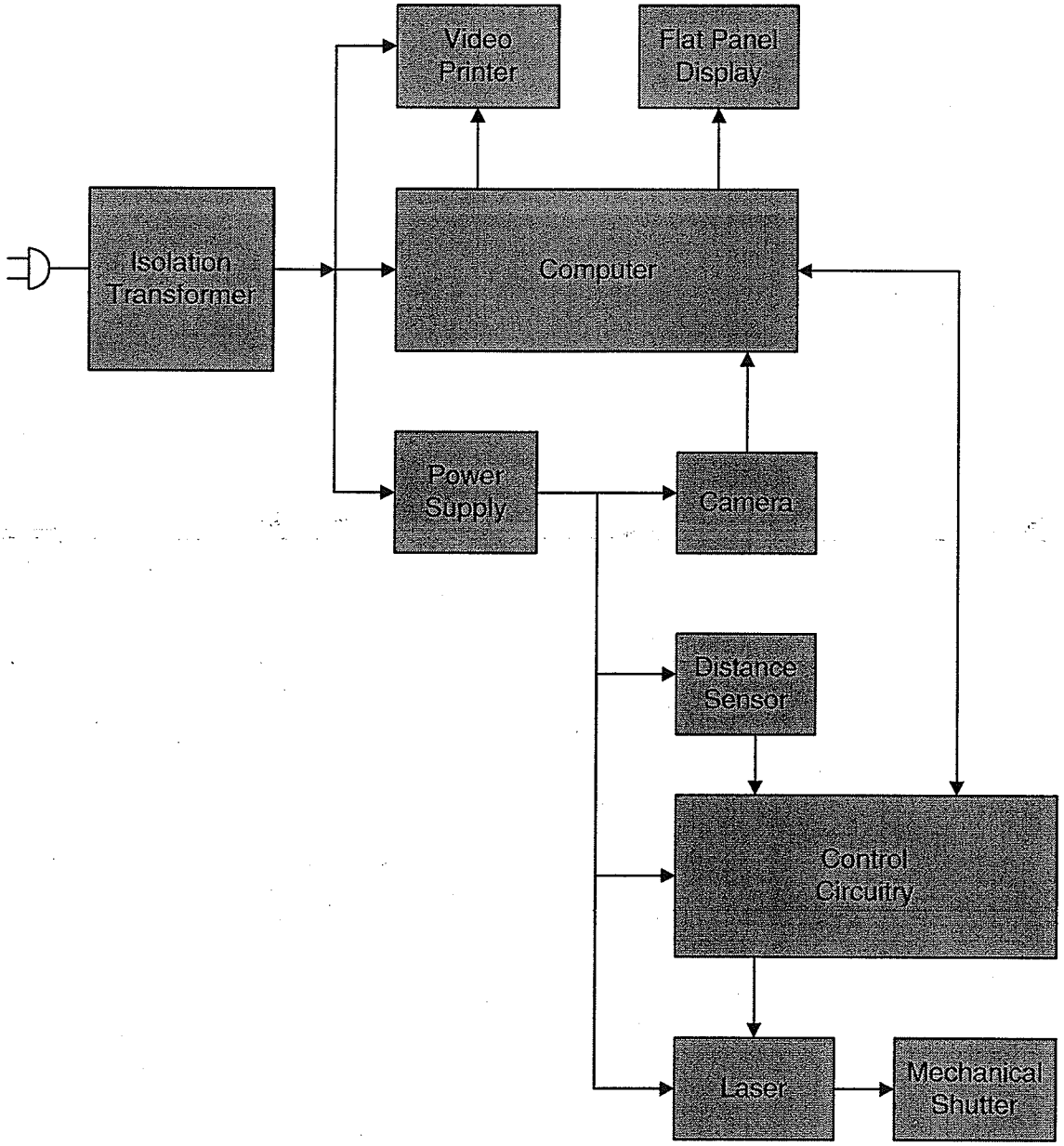
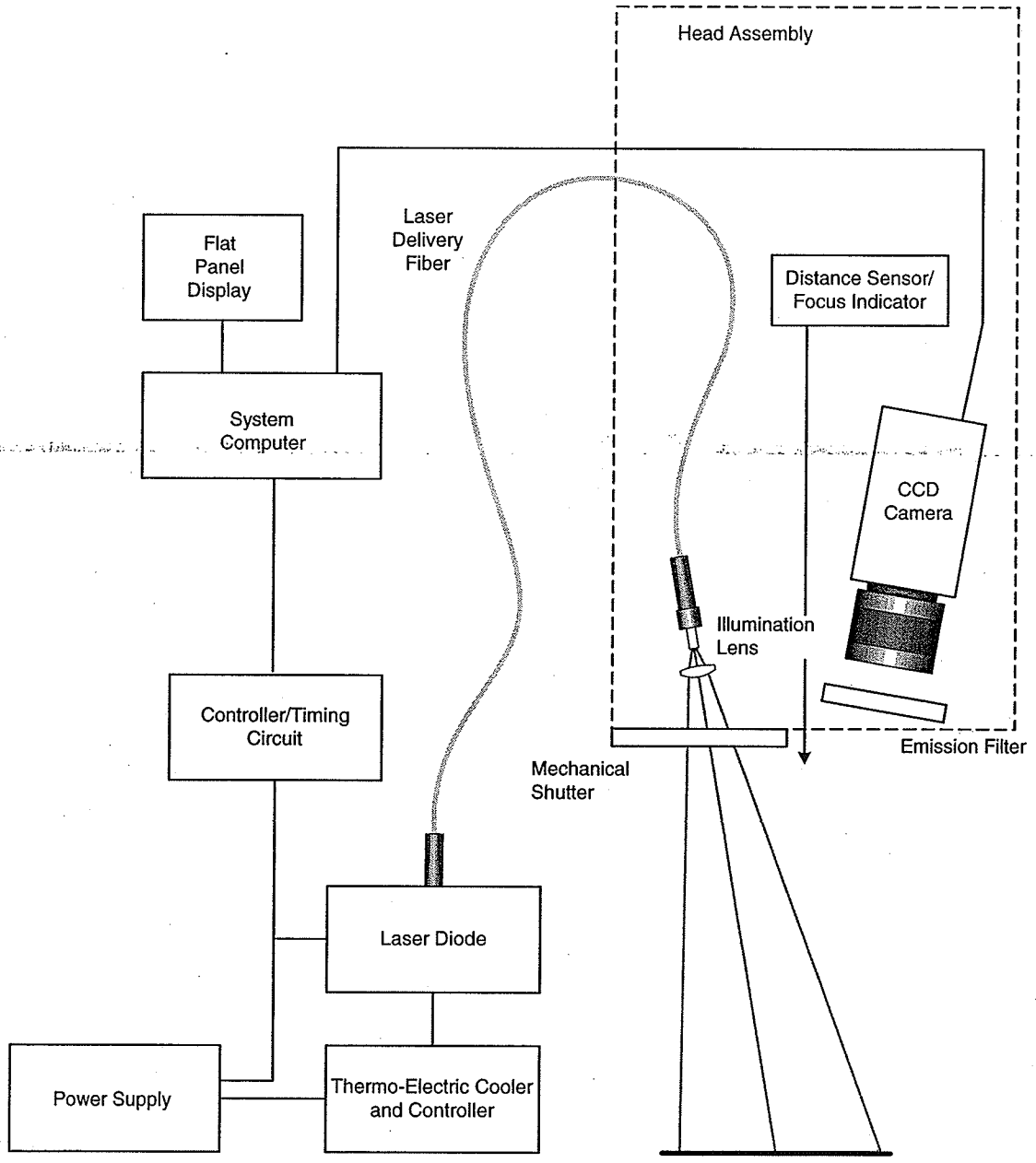


Figure II: Optical Configuration



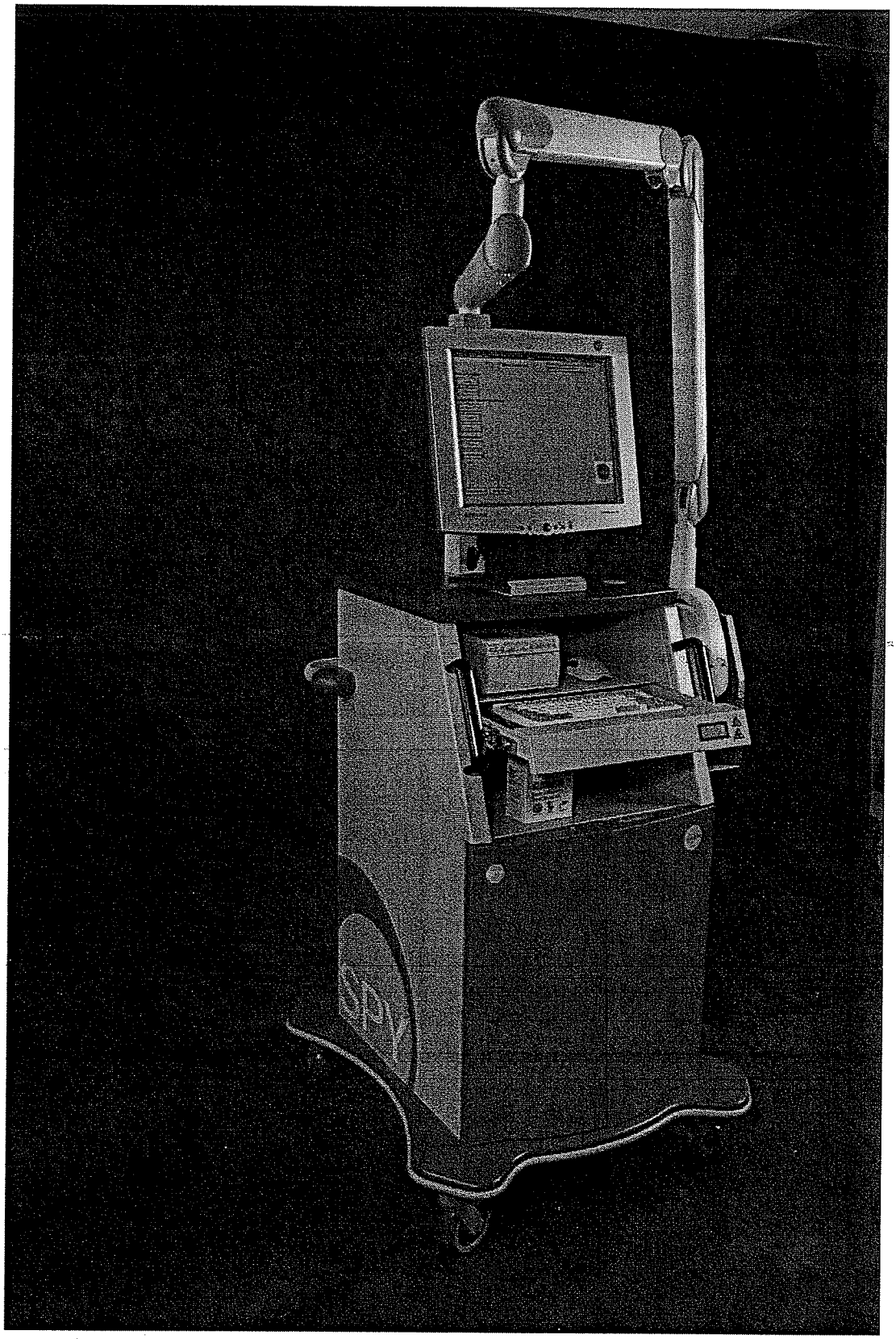


Figure III: SPY 2000 System

CHAPTER VII

REGULATORY APPROVAL

Regulatory approval

In order to allow the developed system to be used in the clinical setting outside of an investigational study it is necessary to obtain regulatory approval in the territories where the device is to be used. For the purposes of this thesis, focus will be on the approval in the United States (Food and Drug Administration (FDA)) which is the most exhaustive process. The approvals for Canada (Health Protection Branch (HPB)) and Europe (CE Mark: through notified body system) will be summarized briefly. Approvals for Europe and Canada were received in 2001 and approval to market the system in the US was received in Jan 2005.

Canada and Europe

In both Canada and Europe the health authorities require that the system is reviewed for efficacy (clinical data) and safety. Both of these regulatory systems focus more heavily on the safety of the device, thereby, leaving the efficacy to be determined in large part by the end-user (surgeon). As such, the device underwent extensive electrical and EMC testing to confirm that it would not cause any harm in the operating room setting. With respect to the drug it was understood by the respective approval bodies that safety data existed for the ICG over a 40 year period and as such it could be approved as part of the system without any further drug trials. The key documentation required by the approval bodies was the following:

(i) Product specification

(ii) System risk analysis

(iii) Design input documents

(iv) Design output documents

(v) Published literature on similar devices

(vi) Instructions for use

(vii) Service manual

(viii) Operators manual

(ix) Assembly manual

(x) Release procedures for commercial production

(xi) Test reports: electrical and EMC

(xii) Shipping report

(xiii) Drug insert and safety data

(xiv) Drug validation and test data

(xv) Labels

(xvi) Clinical data summary

(xvii) Patient information sheet

In addition both the HPB and the notified body (TUV) required that the company that was responsible for manufacturing and selling the device had a stringent quality system (ISO 9001 compliant). Audits of the quality system with the above technical data were required prior to approval and are carried out on an annual basis thereafter. The entire process for approval takes approximately 4 to 6 months after all the above data has been collected and the quality system initiated.

United States: Food and drug administration (FDA)

The process of obtaining regulatory approval for a medical product in the United States involves the designation of the product to one of three groups within the FDA:

(i) Center for Devices and Radiologic Health (CDRH)

(ii) Center for Biologics Evaluation and Research (CBER)

(iii) Center for Drug Evaluation and Research (CDER)

The two potential groups that could review our product were CDER & CDRH. Should CDER be the lead group the approval process is generally much lengthier in that a complete new drug application (NDA) is required which can take up to 36 months to review following submission of all clinical trial data. The trials would require that we prove efficacy and safety of the data with a focus on the actions of the drug (ICG). CDRH has two potential applications that can be submitted, a 510K or a pre-market approval (PMA). With respect to the 510K, the approval process averages only 90 days whereby; we would have to demonstrate that our device is substantially equivalent with respect to safety and indication for use to a product already marketed and approved by the FDA. When a substantially equivalent product is not available, the PMA route is followed which entails an average review period of 12-24 months post submission of all clinical data. As can be seen by reviewing these options the optimal route preferred is the 510K whereby approval is gained in the shortest period of time.

The imaging system developed could not readily be slotted into one of these groups (CDER or CDRH) since it requires both a device and a drug in order to work. For the imaging system, we initially dealt with both CDRH and CDER in order to try and develop a study which would demonstrate the safety and efficacy of the product. The difficulty occurred whereby; CDER, as in most drug trials, was interested in having us look at outcomes as a potential endpoint for the study. The endpoints discussed were repeat surgeries, angina, myocardial infarction and death. In order to obtain statistically

relevant data the number of patients for any one of these endpoints would have been in the thousands and thus not feasible in light of the time to complete and costs. It was possible to convince CDER that a surrogate end point would be more appropriate. CDER maintained through numerous meetings/discussions that we must compare our system to the current gold standard, x-ray angiography which would be used as the surrogate marker whereby, we could either compare our intra-operative angiogram to an intra-operative x-ray angiogram or immediately post-operative x-ray angiogram prior to patient discharge (within 5 days of surgery) This type of study posed the following additional hurdles:

(a) The ability to recruit patients for x-ray angiography is extremely difficult post-surgery since it is an invasive procedure.

(b) Intra-operative x-ray angiography is performed by fewer than 10 centers in North America and as such the time required to complete the study would be greater than 5 years.

Following our inability to develop an appropriate trial which could demonstrate safety and efficacy with CDER we contacted the newly formed Office of Combination Products (OCP) for assistance. The FDA implemented this new group in December 2002. The OCP has been given the authority to make decisions on the jurisdiction of the FDA that will oversee the approval of a product i.e. CDRH, CBER or CDER. The OCP designates one of these three groups as the lead organization in the review process although other groups can be contacted for guidance.

At a meeting with the OCP it was requested that we submit a formal request for designation to their office indicating the evidence as to why the product should be

reviewed by any one specific group. An argument was developed based on the safety and clinical data available at the time and submitted to the OCP. Within 30 days the OCP designated that our product would be reviewed by CDRH who would be the lead group and that only one application would be required i.e. a 510K or PMA. CDRH was contacted and guidance was sought on the type of application required. It was recommended that we seek 510K approval since our system could readily be compared to x-ray angiography systems with respect to indication for use and retinal imaging systems which used ICG for ophthalmic imaging. An application was submitted in October 2004 and approval was obtained in January 2005. As such the device can now be marketed for use in coronary artery bypass surgery in the United States.

CHAPTER VIII

FUTURE APPLICATIONS & CONCLUSIONS

The original goal of this thesis was to develop a system that would allow the measurement of vessel diameters (in vivo) under the influence of varying pharmacological agents. Due to limitations in the ability to develop software that allows the registration of moving images, the goal was not accomplished. However, a system that could provide high quality angiograms was developed. The basic principles of this system were in turn adapted for use in imaging of bypass grafts during coronary artery bypass graft surgery. As such, a clinically useful modality has been developed that could have implications in a number of fields of surgery.

The system developed has been shown to be effective in over 700 patients to date worldwide resulting in revisions in 5-6% of cases. The device has been attributed to saving the lives of several patients and continues to be used routinely on three continents. Approval to sell the device commercially in the United Stated was received in January 2005 and confidential discussions are currently underway with a multinational corporation to implement a full commercial launch of the product in this key market. To date the device has resulted in sales of over $2 million and there is great optimism in regards to its potential commercial success. Publications from leading cardiac centers in the world (Oxford, UK; Zurich, Switzerland; Hiratsuka, Japan; Toronto, Canada) have attested to the capability of the system and it has been recommended by several surgeons that it become the standard of care in coronary artery bypass grafting. As the push towards evidence based medicine continues, it is anticipated that surgeons will be required to validate their work due to the following pressures:

(i) Patient advocacy groups

(ii) Payers: governments, insurance companies etc.

(iii) Legal groups

The invasiveness and cost of the CABG procedure warrants that there is a form of quality control in order to validate that the patient has actually benefited from the procedure. It is my opinion that the SPY system addresses this need and at this time is the best available option in the marketplace.

As the visibility of the system in hospitals has grown, physicians have inquired with respect to developing the device for numerous other applications. Some of these areas of interest are listed below. The technology may be adapted through further enhancements to meet these medical needs and look forward to the challenges of developing these further applications.

(i) Vascular surgery – limbs and carotid arteries

(ii) Neurosurgery

(iii) Plastic surgery – skin flaps

(iv) Organ transplant – liver, kidney etc.

(v) Minimally invasive surgery – through endoscopic application

(vi) Oncology – detection and treatment

The device does have its limitations in that it does not provide quantitative data and only allows imaging of vasculature which is at the surface (or less than 5mm in depth) and in line of sight. These limitations will be the basis of further experimentation and will hopefully be resolved so as to provide the optimal imaging modality.

The process of developing a new technology for the CABG procedure has been a very exciting but arduous task over a period of approximately 6 years. There are a number of factors (efficacy, cost, regulatory approval and manufacturing) that must be balanced in developing a medical device and the process of balancing and attending to all of these tasks was very difficult. The completion of a device which can be used routinely in the clinical setting has involved the assistance of many colleagues at Novadaq Technologies Inc., consultants and the continual support and assistance of my thesis advisor Dr. John Docherty and committee (Dr. Ian Smith, Dr. Wayne Lautt, Dr. Grant Hatch). The success of this project has truly been a team effort and could not have been completed by any one person. It is hoped that the device continues to be used worldwide to improve the care that patients receive and that the experience learned to date can be used to further improve the current device and develop others for use in varying fields of medicine.

CHAPTER IX

REFERENCES

1. Jelinek M. The clinical basis for the management of coronary artery disease. Intern Med J. 2002 Mar;32(3):110-3.
2. Dundar Y, Hill RA, Bakhai A, Dickson R, Walley T. Angioplasty and stents in coronary artery disease: a systematic review and meta-analysis. Scand Cardiovasc J. 2004 Aug;38(4):200-10.
3. Haery C, Sachar R, Ellis SG. Drug-eluting stents: the beginning of the end of restenosis? Clev Clin J Med. 2004 Oct;71(10):815-24
4. Taggart DP. Surgery is the best intervention for severe coronary artery disease. BMJ.2005 Apr;2;30(7494):785-6.
5. Coronary Bypass Surgery and Angioplasty, 1982-1995, Heart Disease and Stroke in Canada, Health Canada (www.hc-sc.gc.ca/hpb)
6. Gardner TJ. Coronary artery disease and ventricular aneurysms. Surgery, Scientific Principles and Practice (Greenfield LJ, Mulholland MW, Oldham KT and Zelnoc GB, eds), 1993;1391-1411.
7. Alderman EL, LevyJH, Rich JB. Analysis of coronary graft patency after aprotonin use: results from the International Multicenter Aprotonin Graft Patency Experience (IMAGE) trial. J Thorac Cardiovasc Surg 1998;116:716-30.
8. Goldman S, Copeland J, Moritz T. Starting aspirin therapy after operation. Effects on early graft patency. Department of Veterans Affairs Cooperative Study Group. Circulation 1991; 84:520-6.
9. Chesebro JH, Clements IP, Fuster V. A platelet-inhibitor-drug trial in coronary artery bypass operations: benefit of perioperative dipyridamole and aspirin

therapy on early postoperative vein graft patency. N Engl J Med 1982; 307:73-8.

10. Fitzgibbon GM, Burton JR, Leach AJ. Coronary bypass graft fate: angiographic grading of 1400 consecutive grafts early after operation and of 1132 after one year. Circulation 1978; 57:1070-4.

11. Taggart DP. Biochemical assessment of myocardial injury after cardiac surgery: effects of platelet activating factor antagonist, bilateral internal thoracic artery grafts, and coronary endarterectomy. J Thorac Cardiovasc Surg 2000;120;651-9.

12. Gundry SR. Discussion of Pfister AJ, Zaki MS, Garcia JM et al. Coronary artery bypass without cardiopulmonary bypass. Ann Thorac Surg 1992; 54; 1092.

13. Subramaniam VA, Sani G, Benetti FJ, Calafiore AM. Minimally invasive coronary artery bypass surgery: a multi centre report of preliminary clinical experience. Ciculation 1995; 92 (Suppl 1), 645.

14. Ancalmo N and Busby JR. Minimally invasive coronary artery bypass surgery: really minimal? Ann Thorac Surg 1997; 64: 928-9.

15. Mack MJ, Osborne JA and Shennib H. Arterial graft patency in coronary artery bypass grafting: What do we really know? Ann Thorac Surg 1998; 66: 1055-9.

16. Schaff HV, Cable DG, Rihal CS, Daly RC, Orszulak TA. Minimal thoracotomy for coronary artery bypass: value of immediate postprocedure graft angiography. Circulation, 1996; 94 (Suppl 1):1-5 (Abstract).

17. Goldstein JA, Safian RD, Aliabadi D et al. Intraoperative angiography to assess graft patency after minimally invasive coronary bypass. Ann Thorac Surg 1998;66:1978-82.

18. Subramanian VA. Less invasive arterial CABG on a beating heart. Ann Thorac Surg 1997;63:S68-71

19. Elbeery JR, Brown PM, Chitwood WR Jr. Intraoperative MIDCABG arteriography via the left radial artery: a comparison with Doppler ultrasound for assessment of graft patency. Ann Thorac Surg 1998;66:51-5

20. Louagie YA, Haxhe JP, Buche M, Schoevaerdts JC. Intraoperative electromagnetic flowmeter measurements in cortonary artery bypass grafts. Ann Thorac Surg 1994;57;357-64.

21. Walpoth BH, Bosshard A, Genyk I et al. Transit time flow measurement for early graft failure during myocardial recvascularization. Ann Thorac Surg 1998;66:1097-100

22. Calafiore AM, Di Giammarco G, Tedoiri G, et al. Left anterior descending coronary artery grafting via left anterior small thoracotomy without cardiopulmonary bypass. Ann Thorac Surg 1996;61:1658-65.

23. Suematsu Y, Takmoto S, Ohtsuka T. Intraoperative echocardiographic imaging of coronary artery bypass grafting with cardiopulmonary bypass. J Thorac Cardiovasc 2001;122:1147-54.

24. Falk V, Walther T, Kitzinger H, et al. An experimental approach to quantitative thermal coronary angiography. Thorac Cardiovasc Surg 1998;46:25-7.

25. Michel F, Duriex M, Levy B, Boulanger CM. Minimally invasive, in vivo exploration of mouse small artery reactivity. J Cardiovasc Pharmacol. 2004; 43(2):271-5.

26. Selke FW et al. Influence of vessel size on the sensitivity of porcine oronary microvessels to nitroglycerin. Am J Phsiol. 1990;258: H515-H520.

27. Tillmans HH et al. Effects of drugs on microcirculation. Microcirculation of the heart. 1982:305-11.

28. Lamping KG et al. Coronary microvascular response to endothelin is dependent on vessel diameter and route of administration. Am J Physiol. 1992;263:H703-H709.

29. Hiller KH et al. Study of microcirculation by coloures microspheres and NMR-microscopy in isolated rat heart: effect of ischemia, endothelin-1 and endothelin-1 antagonist BQ 610. J Mol Cell Cardiol. 1997; 29:3115-3122.

30. Lim JI, Flower RW. Indocyanine Green Angiography. International Ophthalmology Clinics. 1995;35:59-70.

31. Carski TR et al Adverse reactions after administration of indocyanine green. JAMA. 1978; 240: 636.

32. Benya R et al, Reactions to indocyanine green: A case report and a review of the literature. Catheterization and Cardiovascular Diagnosis. 1989; 23-233.

33. Nanikawa R et al A case of fatal shock induced by indocyanine green (ICG) test. Japanese Journal of Legal Medicine. 1978; 31: 209-214.

34. Speich R et al Anaphylactoid reactions after indocyanine green administration, Annals of Internal Medicine.1988;109:345-346.

35. Hope-Ross M et al Adverse reactions due to indocyanine green. Ophthalmology.1994;101: 529-533.

36. Birnbaum Y, Leor J, Kloner RA. Pathobiology and Clinical Impact of Reperfusion Injury. J thromb Thrombolysis. 1997;4(2): 185-195.

37. Kukreja RC, Janin Y. Reperfusion Injury: Basic Concepts and Protection Strategies. J Thrombolysis. 1997;4(1): 7-24.

38. Ravingerova T, Slezak J, Tribulova N, Dzurba A, Uhrik B, Ziegelhoffer A. Free oxyegen radicals contribute to high incidence of reperfusion-induced arrhythmias in isolated rat heart. Life Sci. 1999;65(18-19): 1927-30.

39. Demiryurek AT, Cakici I, Wainwright CL, Wadsworth RM, Kane KA. Effects of free radical production and scavengers on occlusion-reperfusion induced arrhythmias. Pharmacol Res. 1998;38(6): 433-9.

40. Jabr RI, Cole WC. Alterations in electrical activity and membrane currents induced by intracellular oxygen-derived free radical stress in guinea pig ventricular myocytes. Circ Res. 1993;72:1229-1244.

41. Jabr RI, Cole WC. Oxygen Derived Free Radical Stress Activates Nonselective Cation Current in Guinea Pig Ventricular Myocytes: Role of Sulfhydryl Groups. Circ Res. 1995;76:812-824.

42. Abels C., Fickweiler S., Weiderer P, Baumler W, Hofstadter F, Landthaler M, Szeimies RM. Indocyanine Green (ICG) and laser irradiation induce photocoagulation. Arch Dermatol Res. 2000;292(8):404-11.

43. Baumler W, Abels C, Karrer S, Weiss T, Messman H, Landthaler M, Szeimies RM. Photo-oxidative killing of human colonic cells using indocyanine green and infrared light. Br J Cancer. 1999;*0(3-4):360-3.

44. Abels C, Karrer S, Baumler W, Goetz AE, Landthaler M, Szeimies RM. Indocyanine green and laser light for the treatment of AIDS associated cutaneous Kaposi's sarcoma. Br J Cancer. 1998;77(6):1021-4.

45. Fickweiler S, Szeimies RM, Baumler W, Steinbach P, Karrer S, Goetz AE, Abels C, Hofstadter F, Landthaler M. Indocyanine green: intracellular uptake and phototherapeutic effects in vitro. J Photochem Photobiol B. 1997 Apr;38 (2-3):178-83.

46. Shah RR. Drugs QT interval prolongation and ICH E14: the need to get it right. Drug Saf. 2005;28(2):115-25.

47. Williams SG, Silas JH, Joseph F, Jackson M, Rittoo D, Currie P. Troponin T: how high is high? Relationship and differences between serum cardiac markers according to level of creatinine kinase and type of myocardial infarction. Postgrad Med J. 2004;80(948):613-4.

48. Cherrick GR, et al. Indocyanine green: Observations on Its Physical Properties, Plasma Decay and Hepatic Extraction. Journal of Clinical Investigation 39 592-600 (1960)

49. O'Reilly T, et al. Comparison of the Spectrophotometric and High-Performance Liquid Chromatographic Analysis of Indocyanine Green in Estimating Systemic Clearance in Patients with Chronic Liver Disease. Journal of Chromatography 417 190-196 (1987)

50. PaumgartnerG. The handling of indocyanine green by the liver. Schweizerische Medizinische Wochenschrift 1975;5-30

51. Takahashi et al. SPY™: An Innovative Intra-operative Imaging System to Evaluate Graft Patency During Off Pump Coronary Artery Bypass Grafting. ICVTS 2004;3:479-483.

52. Reutebuch et al. Graft occlusion after deployment of the Symmetry Bypass System. Ann Thorac Surg 2003: 75;1626-9.

53. Taggart et al. Preliminary experience with a novel intraoperative fluorescence imaging technique to evaluate the patency of bypass grafts in total arterial revascularization. Ann Thorac Surg 2003;75:870-3.

54. Balacumaraswami et al. Does off-pump total arterial grafting increase the incidence of intraoperative graft failure? J Thorac Cardiovasc Surg 2004: 128:238-44.

55. Reutebuch et al. Novadaq SPY™: Intraoperative quality assessment in off-pump coronary artery bypass grafting. Chest 2004;125 (2):418-24.

APPENDICES

A: DEVICE OPERATORS MANUAL

NOVADAQ TECHNOLOGIES INC.
IMAGING IS EVERYTHING

Novadaq Technologies™ SPY™ Intra-operative Imaging System: SP2000

Operator's Manual

Manufactured by: Novadaq Technologies Inc.
3300 Highway 7, Suite 703
Concord, Ontario
Canada
L4K 4M3

Username	Password
clinical	SPY

Note: FloVision™ is case-sensitive. The username must be entered in LOWER CASE and the password in UPPER CASE.

Novadaq Technologies™ SPY™ Intra-operative Imaging System

DIRECTIONS FOR USE AND SPECIAL INSTRUCTIONS

Carefully read all instructions prior to use. Observe all warnings and precautions noted throughout these instructions.

PRECAUTION:

The Novadaq Technologies SPY™ Intra-operative Imaging System should only be used in an operating room equipped for vascular procedures.

DESCRIPTION:

The Novadaq Technologies SPY™ Intra-operative Imaging System consists of the SPY™ Imaging Device, the Novadrape™ custom sterile drape and Indocyanine Green (ICG) contrast agent. The SPY™ Imaging Device consists of an imaging head containing a Charge Coupled Device (CCD) camera, a laser light source, motion sensor and distance sensor attached via an articulating arm to a mobile cart. The mobile cart contains a flat panel display, computer, electronics enclosure and video printer. The Novadrape attaches to the SPY™ Imaging Device via a specially designed optical window, sterile tapes and is designed to maintain sterility throughout the procedure. The ICG is reconstituted before each procedure, and is administered to patients systemically via a central venous catheter. The SPY™ Imaging System is designed to enable cardiac surgeons to obtain images of the coronary arteries and bypass grafts during coronary artery bypass surgery.

Additional components included in the system are:

a. Flat Panel Monitor
b. Pentium IV Computer
c. Keyboard
d. Optical IntelliMouse
e. B/W video graphic printer

Note: Manufacturers instructions for use and original manuals for the above listed components are available upon request from Novadaq.

LASER CHARACTERISTICS

Wavelength	806 nm
Type	Continuous wave
Duration	34 seconds
Power Output (maximum)	2.4 Watts
Beam Divergence	250 mrad
Area of Illumination	7.6 cm x 7.6 cm
Class	IIIB

CAUTION! Keep eyes out of the direct path of the laser beam and/or specular reflection

The Nominal Ocular Hazard Distance (NOHD) is the distance at which the beam irradiance or radiant exposure equals the corneal Maximum Permissible Exposure (MPE). Using IEC Standard 60825-1:2001 the NOHD for the Novadaq SPY™ system is 27 cm

In order to not exceed the MPE, eyes should not come closer then the NOHD to the laser.

INDICATIONS:

The Novadaq Technologies SPY™ Intra-operative Imaging System is intended for use during coronary artery bypass surgical procedures.

CONTRAINDICATIONS:

Hypersensitivity to indocyanine green or any of its components.

Pregnancy Category C: Animal Reproduction studies have not been conducted with IC-GREEN™. It is not known whether IC-GREEN™ can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. IC-GREEN™ should be given to a pregnant woman only if clearly indicated.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when IC-GREEN™ is administered to a nursing woman.

The SPY™ Intra-operative Imaging System should not be used during coronary artery bypass procedures with patients who are known to be sensitive to iodine.

WARNINGS:

- Do not aim/point the laser directly toward the eyes of anyone involved in the surgical procedure or eyes of the patient.
- Do not place mirrors or highly reflective surfaces in the field of illumination when the laser is activated.
- Do not place hands or other body parts in the path of the laser during use.
- Always operate the SPY™ imaging camera at the proper distance from the field of view as indicated by the range indicator.
- Do not unplug the SPY™ Imaging Device by pulling on the cord.
- Do not use a power cord that shows cracks or abrasion damage.
- Do not hang any objects or materials on the articulating arm.
- The Novadrape™ custom drape is supplied sterile and is intended for single use. Do not resterilize or reuse.
- ICG is supplied in a single vial. Each vial is intended for use with one patient. ICG cannot be stored for future use once it is reconstituted.

- Do not attempt to alter the position, bending radius or attachment of the fiberoptics in the SPY system as this may lead to damage to the fiber with the potential to cause injury to the patient or user.
- **CAUTION – Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure. DO NOT remove or alter cable connections or attempt to remove system components (e.g. printer, monitor, etc.).**

PRECAUTIONS:

- Qualified personnel must install the SPY™ Imaging System in accordance with Novadaq Instructions.
- Use only ICG at indicated doses and concentrations.
- Do not use ICG vials that appear to have seals that are compromised in any way.
- Use only Novadrape™ model ND 3000 sterile drape.
- Do not use drapes in which the seals on the package appear to be compromised in any way.
- Move the imaging head only by grasping the head.
- Move the cart only by grasping the handle with both hands.
- The cart should be moved slowly, otherwise the considerable weight of the device could make it difficult to stop.
- Keep fingers away from "pinch point" areas at the articulations between the mobile arm segments.
- When opening cart door please take caution since items may have moved inside the cart during transport.
- Before using the SPY™ Imaging System, check that the wheels are in the locked position. Test that this is so by applying force to the handle of the cart to ensure immobility of the device.
- Laser equipment not in use should be protected against unqualified use by removal of the key from the KEY LOCK. The key should be kept in a safe place accessible to qualified personnel only.
- Because of potential safety hazards under certain conditions, the preferred use of the SPY™ Imaging System is without an extension cord. However, if one is absolutely necessary for the operation of the device, we recommend the use of a 3-wire cord rated at 10A.

Note

This equipment has been tested and found to comply with the limits for a class A digital device, pursuant to CE. These limits are designed to provide reasonable protection against harmful interferences in a non-residential installation. This equipment generates and uses and may radiate radio frequency energy. If the equipment is not installed and used in accordance with the instructions, the radio frequency energy may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and the receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.

HOW SUPPLIED:

The Novadaq Technologies SPY™ Intra-operative Imaging Device is supplied NON-STERILE and requires no sterilization, as it does not come in direct contact with the patient. The Novadrape™ custom drape is supplied STERILE in an unopened and undamaged package. The Novadrape™ has been sterilized and is for single use only. DO NOT RESTERILIZE. DO NOT REUSE.

ICG is supplied in single use vials. A vial of solvent accompanies each vial of ICG. Each vial of ICG should be reconstituted with a single vial of solvent. Any ICG remaining at the end of a procedure should be discarded.

INSTRUCTIONS FOR USE:

SEE ATTACHED FIGURES A&B FOR CLARIFICATION

1. **POWERING ON AND PREPARING FOR IMAGE ACQUISITION:**

   Insert the device's electrical plug into an appropriate power outlet (100, 120, or 230 volts, depending on the country). The power outlet must be compatible with the plug provided. If the operating room is equipped with equipotential grounding sockets, insert the equipotential plug (green and yellow cord) into the appropriate wall socket.

   Open the cart doors. Turn on the electronics enclosure, which is located on the bottom shelf of the cart. Press the POWER button on the electronics enclosure.

   a. Turn on the SONY UP-895MD printer by pressing the ON/OFF button located on the front, upper left corner of the device. Ensure that it is turned on before turning on the computer.

   b. Depress the power button on the front of the computer. This will cause both the computer and monitor to power on.

   c. At the login window, enter the USERNAME and PASSWORD provided at the front of this manual. The FloVision software will open automatically, displaying the MAIN MENU.

   d. At the FloVision™ Main Menu, under the heading CLINICAL FUNCTIONS select ACQUIRE (see Figure 1).

Note: If 5 or more patients have already been stored on the computer's hard drive, the user will be warned to backup data to CD (see Figure 2a)

Note: If FloVision™ detects a shortage of disk space on the hard drive, the user will be warned to immediately archive patient data to CD (see Figure 2b)

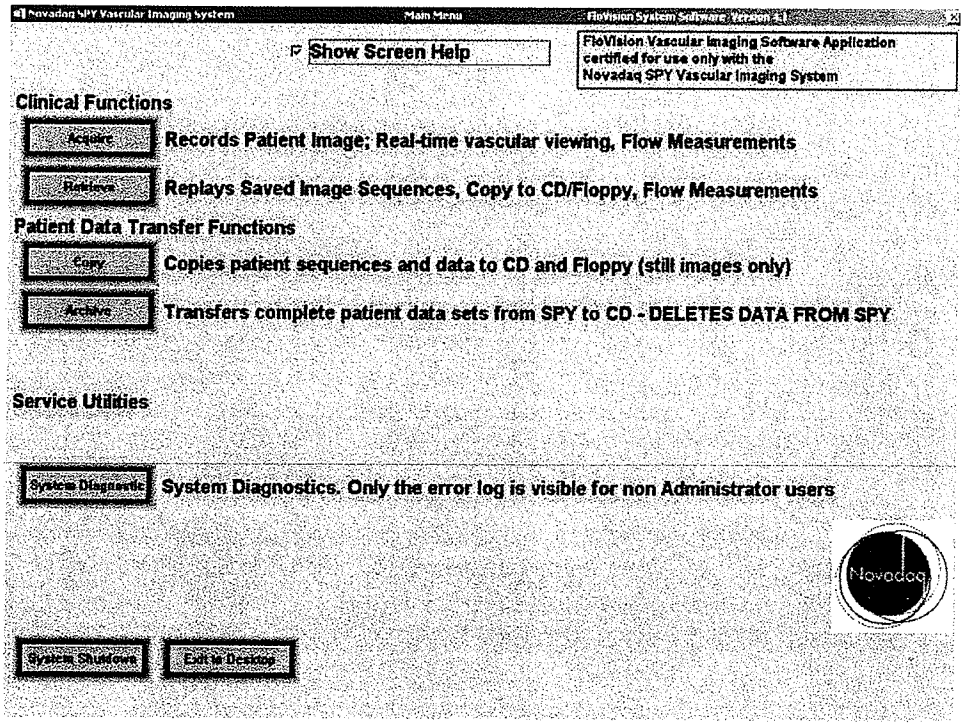


Figure 1

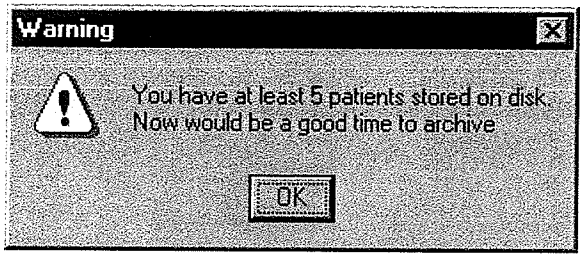


Figure 2a

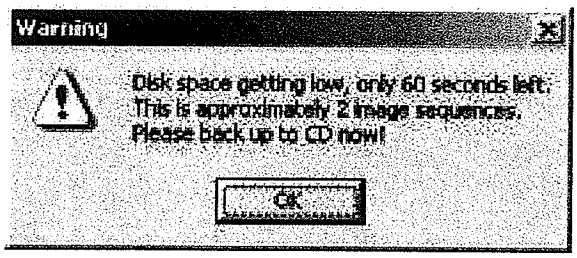


Figure 2b

e. If this is a new patient, enter patient data (Name and identification number) in the box on the right side of the screen. Press CONTINUE to proceed. If this is a patient being imaged for a second or subsequent time, select the patient's name from the box on the right of the screen (see Figure 3).

Note: If the user fails to enter the patient's information correctly, the following Error pop-up windows will appear upon clicking the CONTINUE button (see Figures 3a-g):

- **Figures 3a, 3c: if the user does not enter the patient's last and first names, respectively.**
- **Figures 3b, 3d, 3e: if the user enters an invalid character.**
- **Figure 3f: if the user does not enter the patient's ID or enters it improperly (e.g. with invalid characters).**
- **Figure 3g: if any of the patient information fields are not properly completed.**

f. A pop-up screen will ask you to confirm that the patient data entered are correct. If the data are correct press YES, otherwise, select NO and correct the patient data (see Fig. 4a). This will take you to the ACQUIRE- Preview screen (see Figure 5). The image field on this screen displays a real time image.

Locate the switch for turning the IR LED CONTROL on and off, on the imaging head. With the switch in the OFF position check that the image field on the monitor displays a black background. Turn the IR LED switch ON and check that the monitor displays an illuminated image.

2. APPLYING THE STERILE NOVADRAPE™:

Note: Unless otherwise indicated, the following steps in the application of the Novadrape are to be performed by the sterile operator.

a. The un-sterile operator extends the imaging head of the unit by pulling it away from the cart.

b. Using proper sterile technique, open the package labeled Novadrape™ sterile drape.

c. Place your hands inside the fold of the open end of the Novadrape™.

d. Continuing to hold the Novadrape in this manner, place the Novadrape™ over the imaging head and distal arm of the unit.

e. As the drape is passed over the imaging head and distal arm, the un-sterile operator grasps the drape by the un-sterile portion of the interior of the drape that has been folded over and pull the drape over the remainder of the arm.

Note: The Novadrape™ should cover the arm up to and including the horizontal bar.

Note: The Novadrape™ is equipped with an optical interface, which is to be positioned directly over the apertures of the imaging head.

f. Bring the optical interface of the Novadrape™ into alignment with the rectangular window of the imaging head.

g. Attach the optical interface to the imaging head by snapping the rim of the optical interface onto the rectangular window.

h. The un-sterile operator pulls the Novadrape™ tightly over the articulating arm.

i. Pull the fastening ties tightly around the arm to ensure that the Novadrape is attached securely.

Note: If the Novadrape should become contaminated at any time during the procedure, it should be replaced with a new sterile drape and applied to the SPY™ Imaging Device in the manner outlined above.

Figure 3a

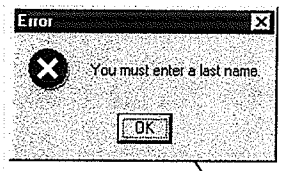


Figure 3b

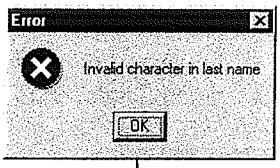


Figure 3c

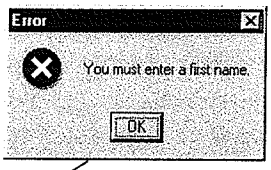
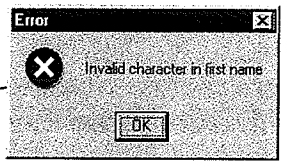


Figure 3d



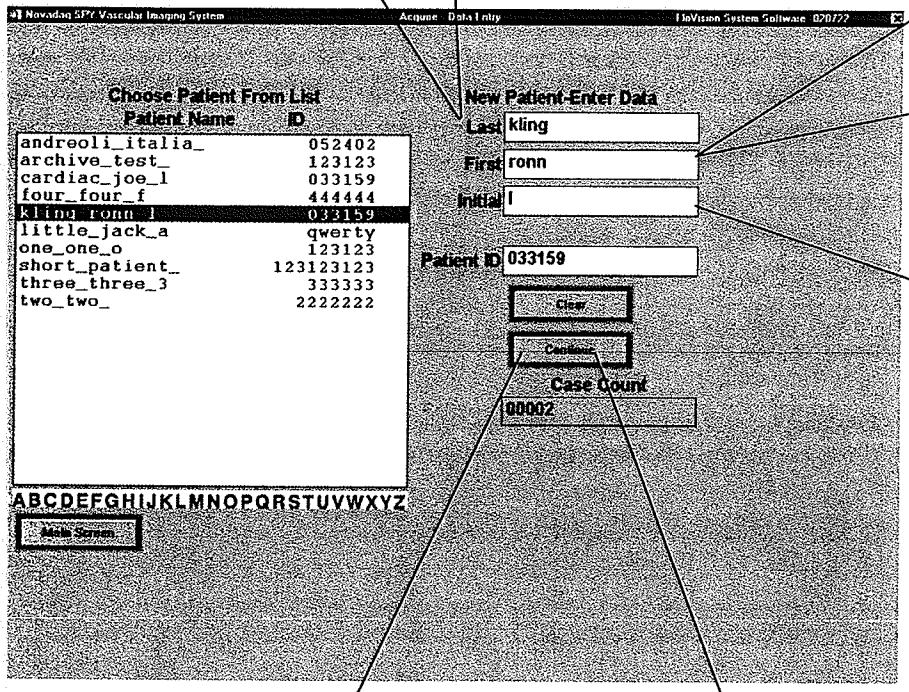


Figure 3e

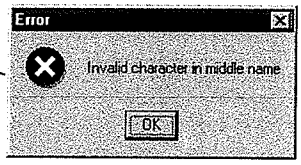


Figure 3

Figure 3g

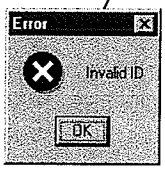
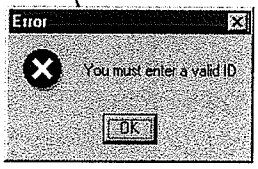


Figure 3f



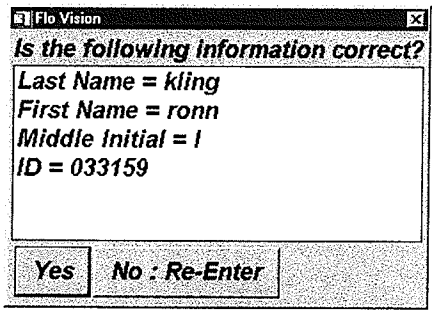


Figure 4a

Note: Before proceeding, the user will be reminded to turn the laser key to the horizontal ('on') position (see Figure 4b)

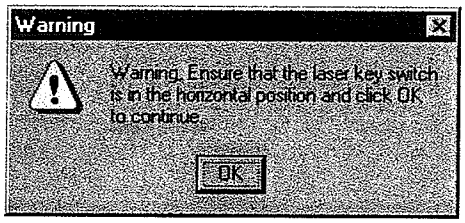


Figure 4b

3. ENABLING THE SPY™ IMAGING DEVICE LASER:

a. Insert the key that is provided into the key-lock on the electronics enclosure located on the bottom shelf of the cart.

b. Turn the key lock switch to the horizontal position to enable the laser.

Note: T his does not cause the laser to illuminate but rather allows the laser to activate upon pressing the RECORD button.

c. Check the position of the LASER EMERGENCY STOP.

Note: The LASER EMERGENCY STOP is located on the top of the imaging head and provides the user with a mechanical means of interrupting the laser output in the event of an emergency. It is the large red colored button located on the top half of the imaging head (see FIGURE B).

d. Verify that the LASER EMERGENCY STOP is in the pulled out position.

SPY Imaging Device

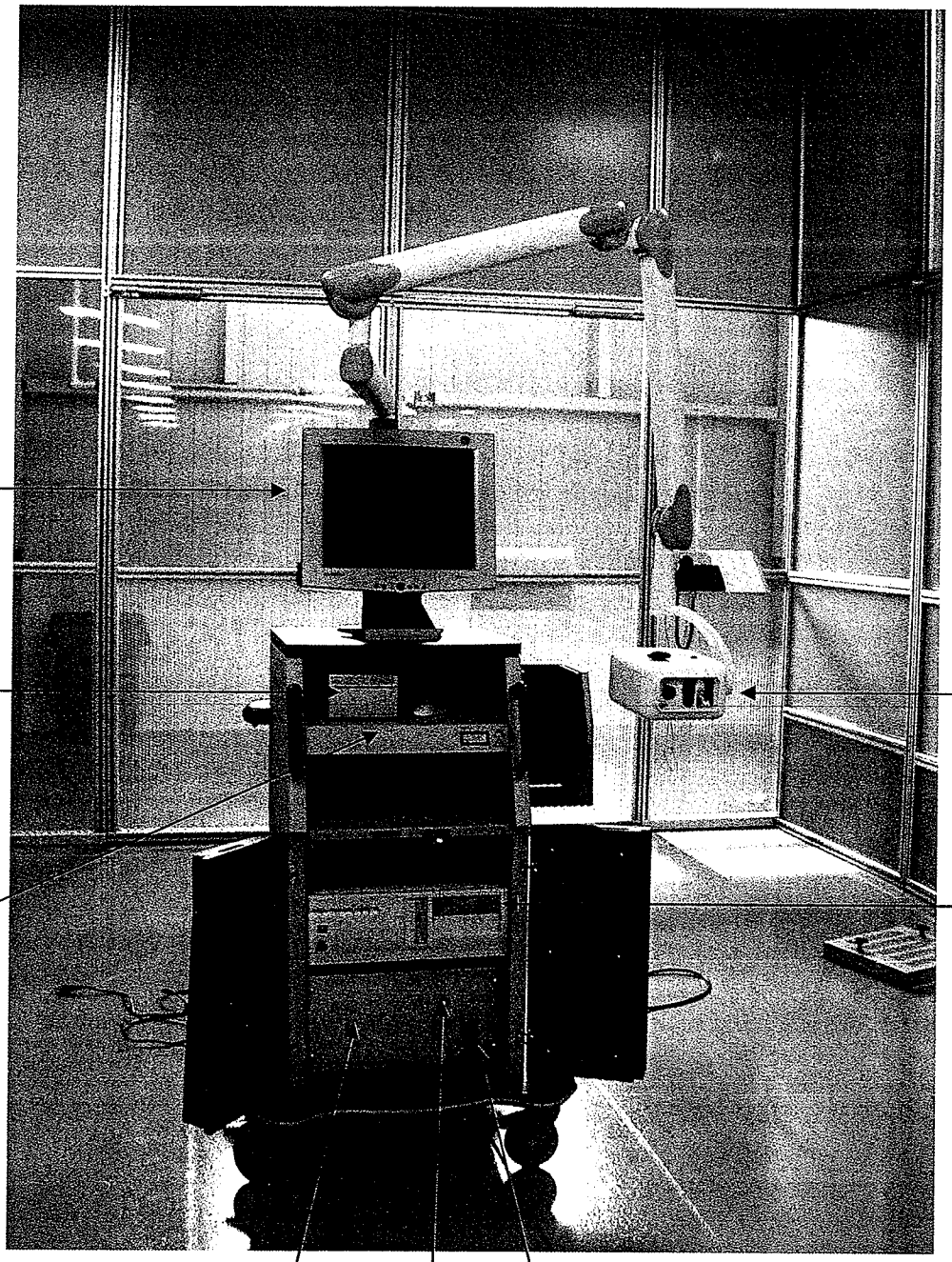


FIGURE A

Imaging Head Components

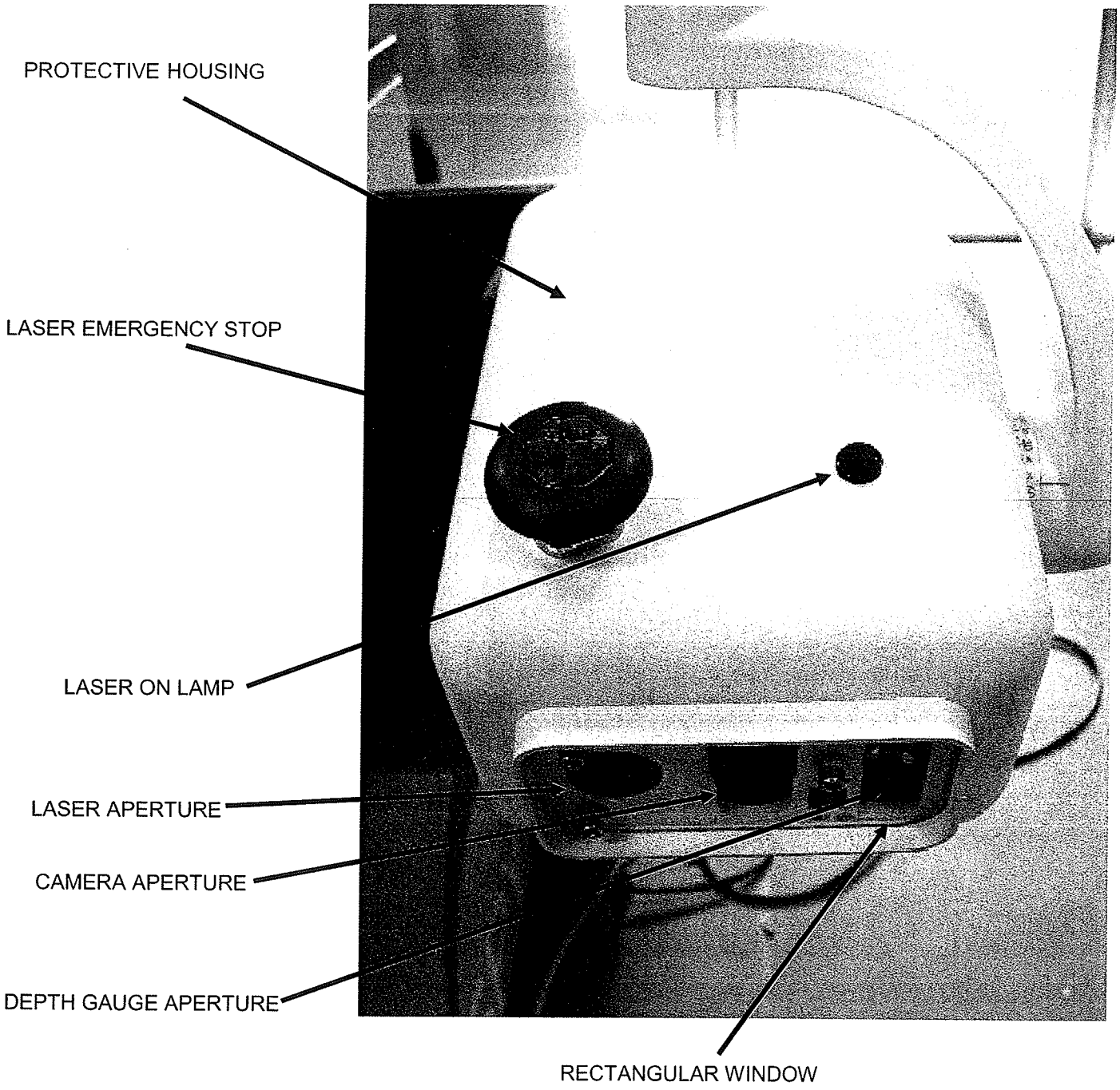


FIGURE B

4. Reconstituting the Indocyanine Green (ICG) for use with the SPY™ Imaging Device:

Note: The ICG kit consists of lyophilized ICG along with the sterile aqueous solvent.

a. Using proper mixing technique, inject 10ml of the sterile aqueous solvent into the vial containing the indocyanine green (25 mg) and shake gently to mix.

5. PREPARATION OF THE VENOUS ACCESS LINE FOR ICG ADMINISTRATION:

Note: Indocyanine green administration is to be performed through the central intravenous line.

a. Configure the central venous access line so two injection ports are available for the administration of ICG.

6. ACQUIRING IMAGES

a. An un-sterile operator moves the complete device into position. A sterile operator then positions the imaging head over the operating field.

Note: Positioning of the SPY™ IMAGING device should be done by pushing on the cart only. Do not attempt to move the device by pushing or placing force on the monitor or the articulating arm.

b. Ensure that the IR LED control switch is on and that a real time image can be observed on the monitor. This will aid in selecting the appropriate view of the heart.

The SPY™ system incorporates a range detector to ensure the imaging head is at the correct focal distance from the heart. The laser will not activate if the imaging head is not within 30-±2.5 cm. While positioning the imaging head approximately 30 cm from the heart, observe the RANGE on the right side of the monitor. Ensure that the arrows on the range indicator are in line with the green region

c. When the system is ready to record the READY button will illuminate green. The laser cannot be activated unless the READY indicator has turned green (see Figure 5).

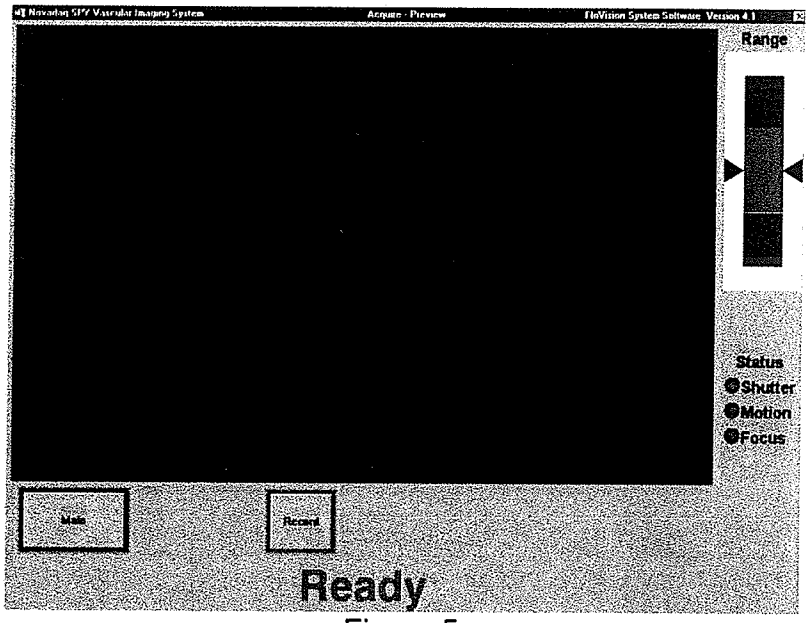


Figure 5

d. When the appropriate view of the heart has been obtained and the READY indicator is green, turn off the IR LED by depressing the rocker switch on the imaging head.

Note: The system has a motion detector in the head, and will not allow the laser to be activated if the head is in motion. The detector will also shut the laser off if the imaging head is "bumped" while the laser is activated. Therefore, the imaging head should not be moved during imaging.

Note: Optimal image quality requires that prior to imaging, the background be as dark as possible. Turn the surgical lights away from the heart to ensure a dark background. Do not start image acquisition until the background of the image on the monitor is as dark as possible.

e. Inject 1.0 mL of ICG in a rapid bolus followed by a rapid injection of 5mL of saline through the central venous line.

f. At the same time, activate the laser and start acquisition to the computer by clicking RECORD on the ACQUIRE – Preview screen.

Note: An Error pop-up window will appear and inform the user that the software is returning to the Preview screen should any of the following malfunctions occur during an image acquisition sequence – should multiple problems occur, more than one error message will be displayed in a single window (Figs. 6a-h):

- **If the Laser Emergency Stop/Shutter button is pushed (Figure 6a)**
- **If the imaging head is moved suddenly (Figure 6b)**
- **If the laser temperature goes too high or too low (Figures 6c & 6d)**
- **If the LASER ON LAMP goes out (Figure 6e)**
- **If the system is not OK (Figure 6f)**
- **If the laser power moves too high or too low (Figure 6g & 6h)**

Press 'OK' to continue.
IF THE PROBLEM PERSISTS, PLEASE CALL YOUR DISTRIBUTOR.

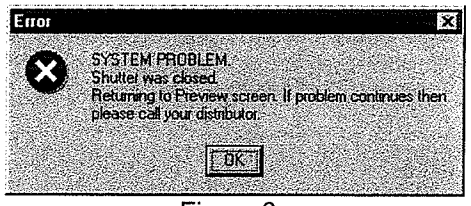


Figure 6a

Figure 6b

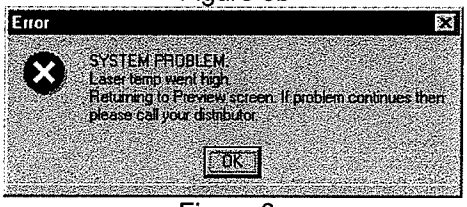


Figure 6c

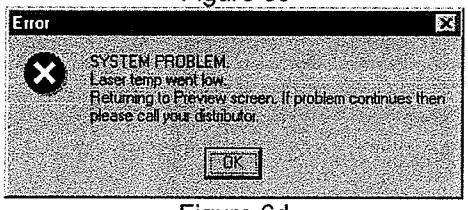


Figure 6d

Figure 6e

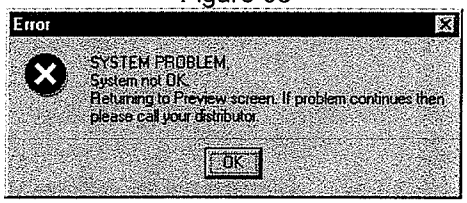


Figure 6f

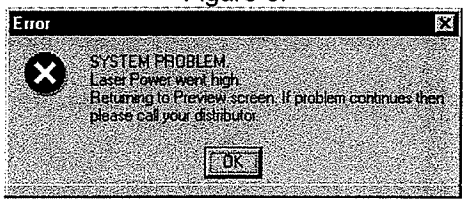


Figure 6g

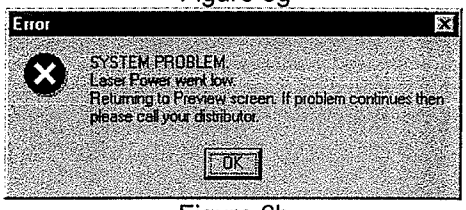


Figure 6h

g. Upon activation of the laser, the two amber LASER ON lamp indicators will illuminate. The LASER ON lamp indicators are located on the top and bottom of the imaging head.

h. The LASER ON indicators will be illuminated for the entire time that the laser is activated.

i. The laser will automatically deactivate after 34 seconds. Image acquisition to the computer will also automatically stop after 34

seconds. At this point, an Information pop-up window will appear informing the user that the laser has been turned off and that the FloVision™ software is advancing to the ACQUIRE – SAVE screen (see Figure 7).

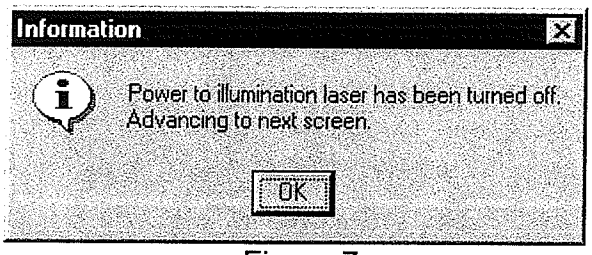


Figure 7

7. MANUAL LASER DEACTIVATION/ STOP:

a. A manual STOP/REVIEW button is located directly below the image-viewing field. Pressing the STOP/REVIEW button (see Figure 8) immediately deactivates the laser, stops image acquisition to the computer and transfers to the ACQUIRE-SAVE window (see Figure 9). Pressing the MAIN button while recording causes a Question pop-up window to appear informing the user that the current image sequence will be lost and asking them if they wish to continue (see Figure 8a). Click YES to proceed and NO to record another sequence.

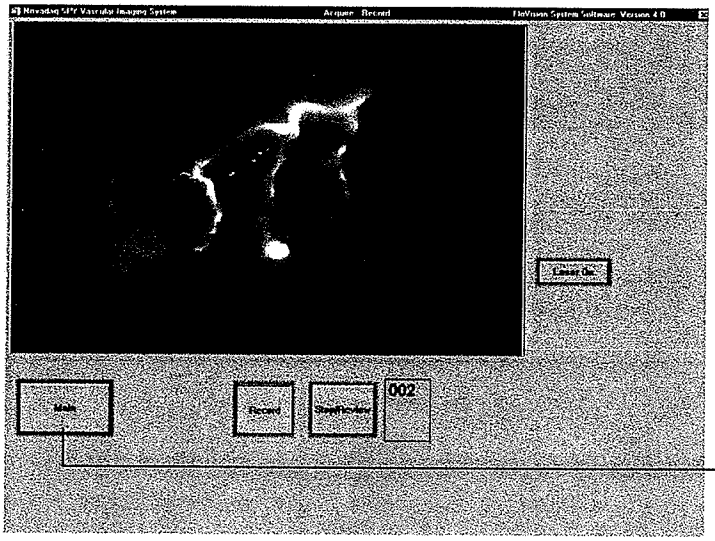


Figure 8

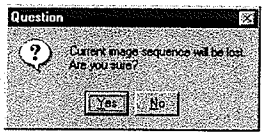


Figure 8a

8. SEQUENCE REPLAY AND SAVE:

NOTE: AT THIS TIME THE IMAGES ARE STORED IN THE COMPUTER'S RAM AND HAVE NOT BEEN PERMANENTLY SAVED TO DISK. THE ACQUIRE-SAVE SCREEN IS USED TO SAVE IMAGES TO THE COMPUTER HARD DISK.

IT IS IMPORTANT THAT YOU DO NOT SAVE EXCESSIVELY LONG IMAGE SEQUENCES AS THIS WILL INCREASE THE TIME NEEDED TO STORE AND RETRIEVE IMAGE SEQUENCES AND WILL INCREASE THE AMOUNT OF STORAGE AND ARCHIVE SPACE REQUIRED. THE SPY™ SYSTEM ALLOWS YOU TO ACQUIRE 34 SECONDS OF IMAGES IN ORDER THAT NO IMPORTANT DATA BE MISSED. HOWEVER IT IS UNLIKELY THAT ALL OF THE IMAGES MUST BE SAVED TO DISK. SELECT ONLY IMAGES THAT SHOW THE FILLING OF ICG INTO CORONARY ARTERIES AND GRAFTS AND THE WASHOUT PHASE

a. The total number of images acquired is displayed in the top right corner of the ACQUIRE – SAVE screen (see Figure 9).

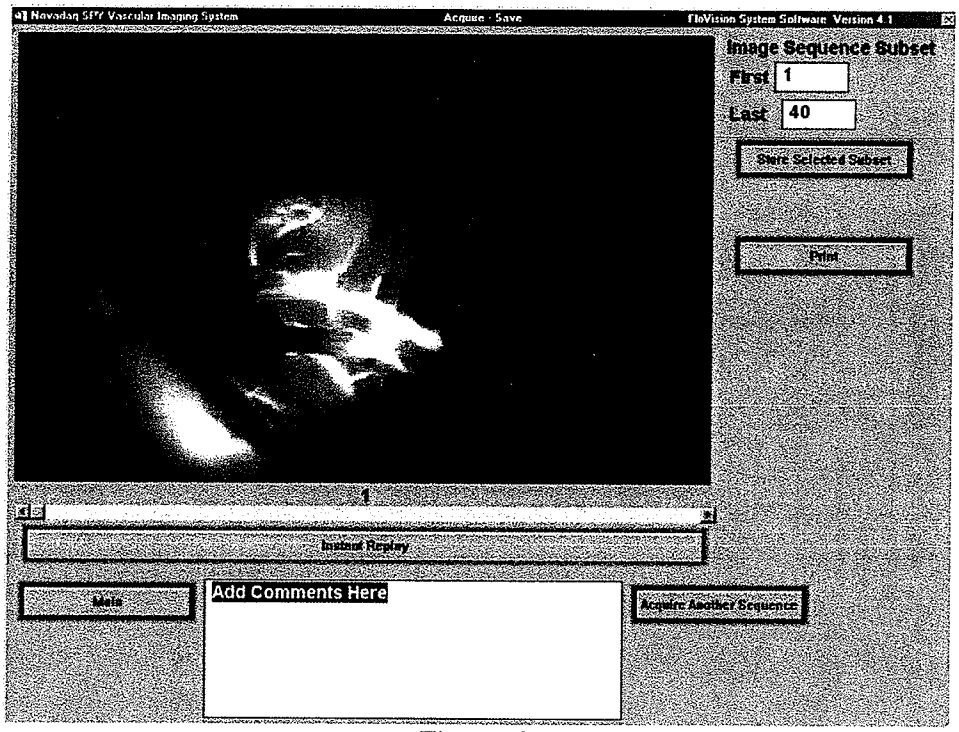


Figure 9

b. Depress the INSTANT REPLAY button to review the image sequence. Use the scroll bar at the bottom of the image field to manually advance through an image sequence. The number of the currently displayed image is shown above the scroll bar.

c. While in the INSTANT REPLAY function select the range of the image sequence to be saved. Enter the first and last image number in the boxes provided under IMAGE SEQUENCE SUBSET.

Note: If the Image Sequence Subset numbers entered are not specified correctly, the user will receive a Warning pop-up window (see Figure 10).

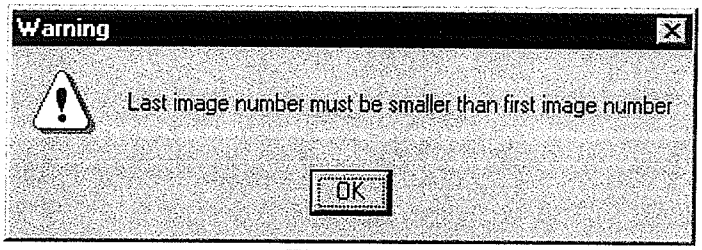


Figure 10

d. In the COMMENTS window, add comments that will assist during later retrieval/analysis of the sequence (e.g. the name of the conduit and the coronary artery to which it is grafted).

Note: If no comments are entered, the user will be presented with a 'Question' pop-up window asking them whether they want to enter any comments before continuing. Click 'Yes' to enter comments; otherwise, click 'No' (see Figure 11).

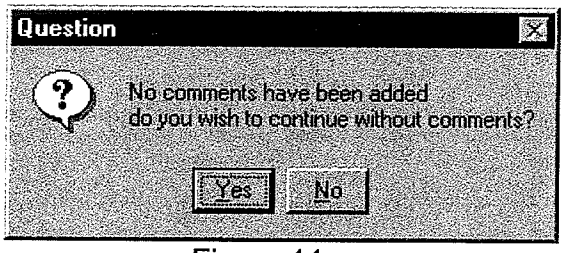


Figure 11

e. To SAVE the sequence (and the patient orientation marker) press STORE SELECTED SUBSET.

f. To acquire another image sequence press ACQUIRE NEW SEQUENCE.

Note: If you do not save before acquiring a new sequence, a 'Question' pop-up window will appear stating that the original subset of images has not been saved and will be lost upon proceeding. To proceed anyway, click YES; otherwise, click NO (see Figure 12).

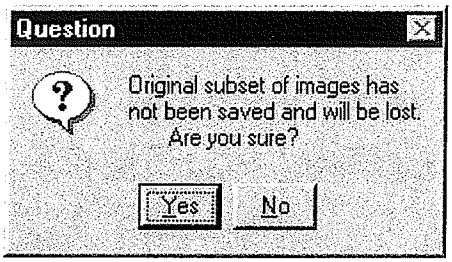


Figure 12

g. If this is the final sequence to be acquired press MAIN.

Note: If a problem exists with the imaging board, the user will be informed with one of two possible Warning pop-up windows upon pressing the MAIN button while in the ACQUIRE – SAVE screen (see Figure 13). Should this occur, please contact your distributor immediately.

Figure 13

9. Printing a Single Frame

A single frame from the sequence can be printed on the printer provided.

a. From the MAIN menu under CLINICAL FUNCTIONS select RETRIEVE (See Figure 1).

b. In the RETRIEVE – SELECT screen select the patient name from the panel on the left (see Figure 14).

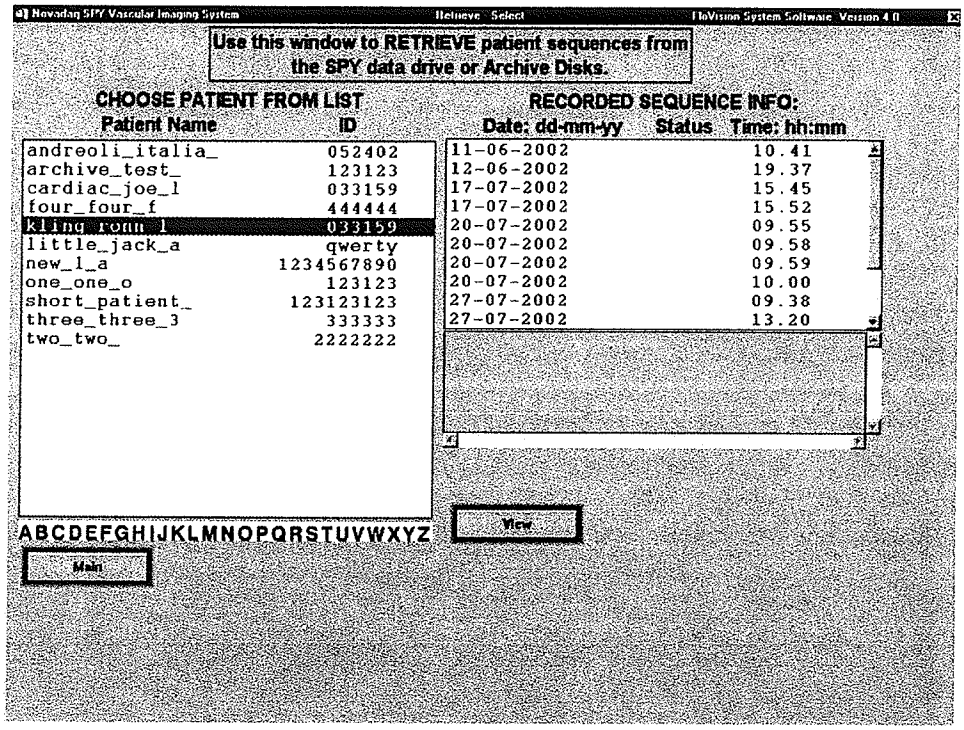


Figure 14

c. From the panel on the right select the sequence of interest.

d. When the sequence has completed loading, view the sequence by pressing PLAY (▸). Stop the sequence at the frame that you wish to print (see Figure 15).

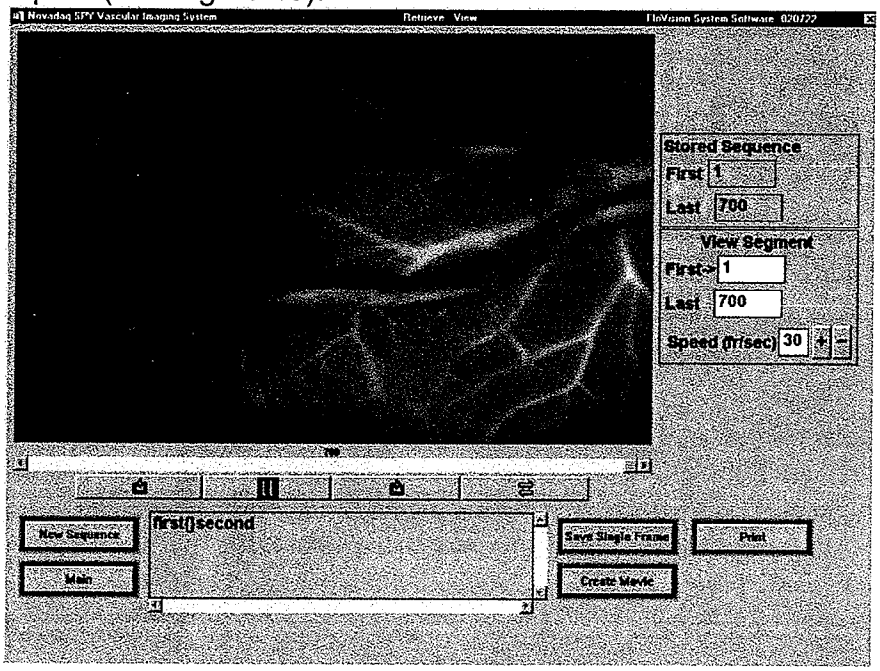


Figure 15

e. Press PRINT – the image will expand to fill most of the screen.

f. Press the PRINT BUTTON on the printer – the image on the screen will print out.

g. To shrink the image back to size, click on the screen outside of the image area.

10. POWERING DOWN, REMOVING THE DRAPE AND STORING THE DEVICE:

From the MAIN MENU select SYSTEM SHUTDOWN. The computer will automatically power down. Once the computer has completely powered down, turn off the SPY™ imaging device by switching the POWER button off.

a. Upon completion of the procedure pull the unit away from the operating field. Once clear of the field, the sterile drape can be removed.

NOTE: UPON COMPLETION OF THE SURGICAL PROCEDURE THE STERILE DRAPE IS LIKELY TO BE CONTAMINATED WITH HUMAN BLOOD. THE DRAPE SHOULD ONLY BE HANDLED AND REMOVED BY PERSONEL WEARING APROPRIATE PROTECTIVE GLOVES. THE DRAPE SHOULD BE DISPOSED OF IN ACCORDANCE WITH YOUR HOSPITAL PROCEDURE FOR DISPOSAL OF LIGHTLY CONTAMINATED MATERIALS.

b. The arm can be folded to its storage position and secured within the protective casing attached to the side of the cart.

c. The laser KEY LOCK can be turned to the off-position and removed for storage.

d. The unit can be unplugged from the power outlet and rolled to its storage position.

11. COPY

The image sequences saved on the SPY™ system are in AVI format. These sequences may be played on any PC (desktop or mobile) that has the appropriate media player installed. To make a copy of a sequence for playback on another computer or for sharing with other people use the COPY function

a. Insert a blank CD into the CD drive of the SPY™ device.

b. From the MAIN menu under PATIENT DATA TRANSFER FUNCTIONS select COPY (see Figure 1).

c. From the panel on the left in the COPY – SELECT window, CHOOSE PATIENT FROM LIST, click on the name of the patient whose sequences will be copied (see Figure 16).

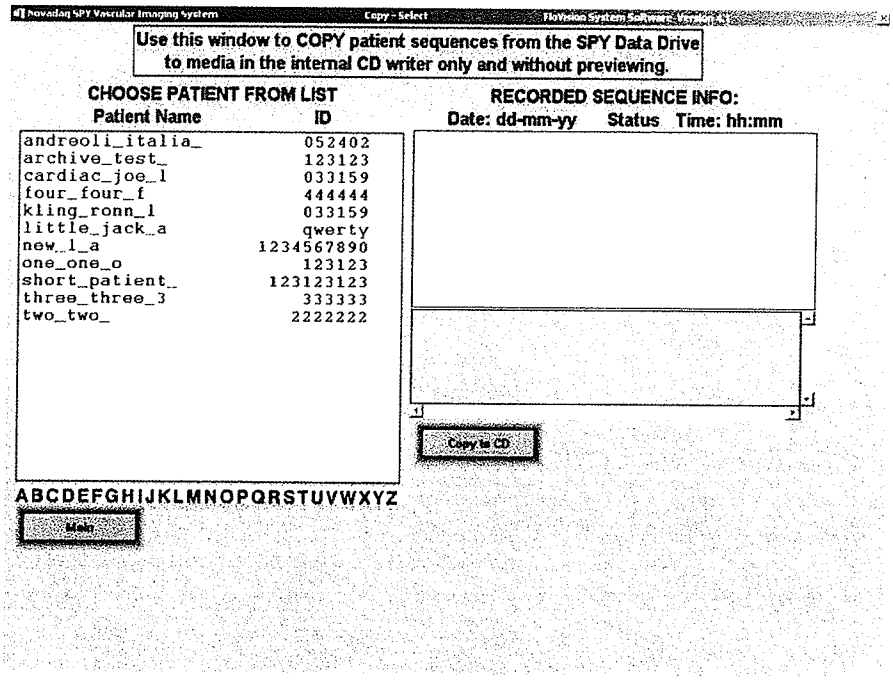


Figure 16

d. The panel on the right of the screen will display the sequences recorded for the patient selected. Highlighting a recorded sequence will display the comments associated with that sequence. This will aid in selection of appropriate sequences for copying. Select a sequence for copying and then press COPY TO CD.

Note: If a patient's information has already been archived it will no longer be available on the computer's hard drive and the user will receive a Warning pop up window when the COPY TO CD button is selected (see Figure 17a). If a communication error occurs between the computer and the CD drive during copying, the user will be warned immediately with another pop-up window (see Figure 17b).

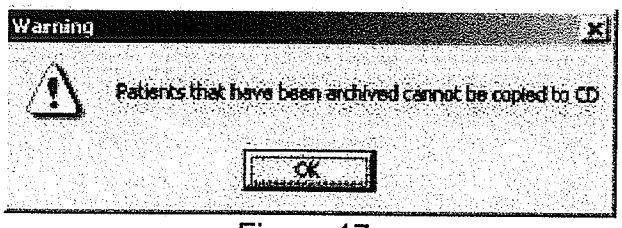


Figure 17a

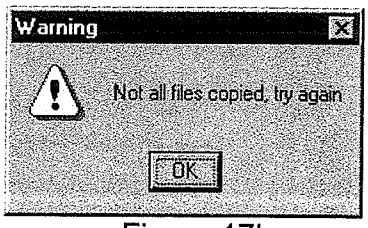


Figure 17b

e. If multiple sequences are to be copied, select them by clicking on each individual sequence while holding the CTRL key.

f. When copying is complete, the CD will automatically be ejected.

g. Press MAIN to return to the main menu.

12. ARCHIVE

Please note that the image sequences are very large files and will rapidly fill up the capacity of the computer hard drive. Patient images must be removed from the hard drive and transferred to archive media at regular intervals to prevent the hard drive from filling up. Failure to archive images could result in a situation where no hard drive space is available for image acquisition.

NOTE – USE OF THE ARCHIVE FUNCTION PERMANENTLY REMOVES IMAGE SEQUENCES FROM THE SPY™ SYSTEM.

a. Insert a CD into the CD drive of the SPY™ device.

b. From the MAIN menu under PATIENT DATA TRANSFER FUNCTIONS select ARCHIVE (see Figure 1).

c. From the panel in the ARCHIVE window click on the name of the patient whose sequences will be archived - use the alphabet scroll bar at the bottom of the list for ease of navigation through the patient list (see Figure 18).

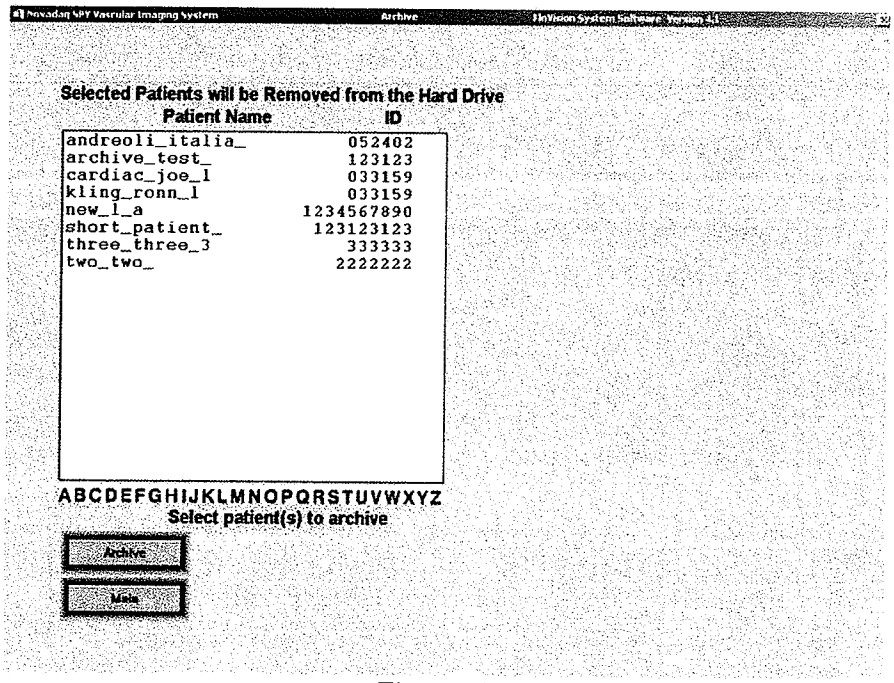


Figure 18

d. Press ARCHIVE.

e. When archiving is complete, press MAIN to return to the main menu.

Note: A Warning pop-up window will appear if there is either no CD in the CD drive (see Figure 19a) or if there is insufficient storage space on the CD in the drive (see Figure 19b). Click 'OK' to continue and try opening and closing the drive or replacing the CD (if necessary)

Figure 19a

Figure 19b

Note: If a communication error occurs between the computer and the CD drive during copying, the user will be presented with one of two possible pop-up windows indicating that either not all files were copied (see Figure 20a) or that one of the files was not copied correctly (see Figure 20b).

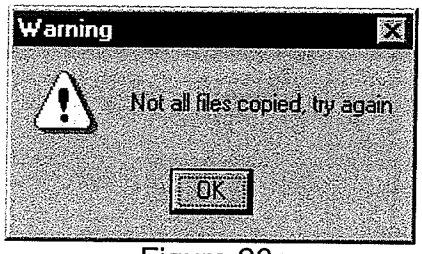


Figure 20a

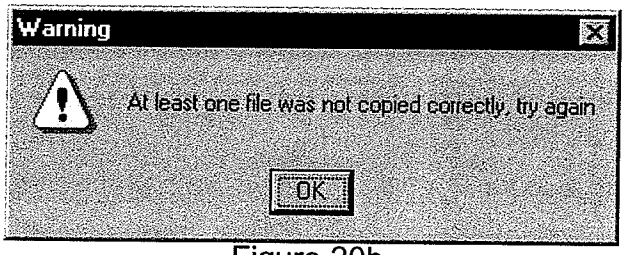


Figure 20b

f. When archiving has completed successfully, eject CD from CD drive.

13. SYSTEM DIAGNOSTICS

Potential problems with the SPY™ device may be assessed using the SYSTEM DIAGNOSTIC tool located on the MAIN MENU under the SERVICE UTILITIES command subset. Here the user can assess minor problems that are interfering with the acquisition of an image sequence (e.g. closed shutter, movement of the imaging head) or more serious issues such as those that may occur with the laser diode.

a. On the FloVIsion™ MAIN MENU, under the SERVICE UTILITIES command set, click on the SYSTEM DIAGNOSITICS button. This will load the SYSTEM DIAGNOSTIC window (see Fig. 21).

b. Select the ACTIVATE DIAGNOSTICS button located at the upper left-hand corner of the screen.

Note: At this point, the distance between the imaging head window and its target will be displayed in the Range field. The temperature of the laser diode will be displayed immediately below this box and the power level will appear above. A problem with any of the device's functions generates an ERROR CODE letter and an accompanying BINARY STRING number (1s and 0s) that appear in adjacent display boxes beneath the TEMPERATURE reading.

Note: Normal Status Indicators initially appear red in the SYSTEM DIAGNOSTIC window; Abnormal Status Indicators are black. When ACTIVATE DIAGNOSTICS is selected, the Normal Status Indicators turn green when the systems that they monitor are functioning properly and red when there is a problem. The Abnormal Status Indicators appear white when the systems they monitor are functioning properly and black when they are not.

c. Click on the ACTIVATE LASER button. This will turn on the laser and allow the user to monitor various device functions.

Note: At this point, the Power level should be approximately 2.0 watts. The laser temperature should cycle between 12°C and 14°C

d. If an error occurs with any of device functions that are monitored during laser activation, the appropriate error code letter and binary string will appear in their respective display boxes (see below).

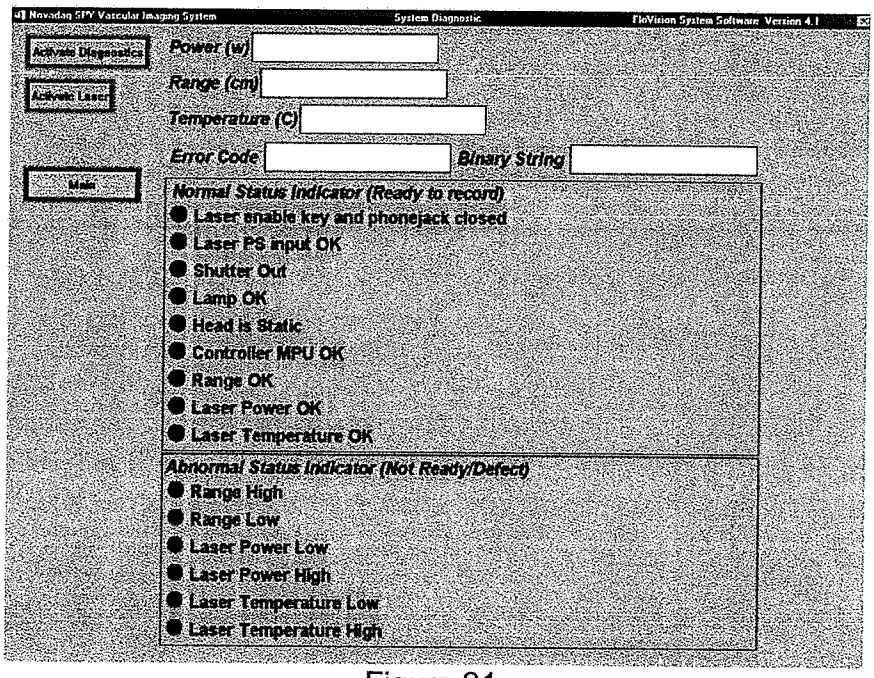


Figure 21

FLOVISION ERROR CODES

A = laser not run

B = laser on

F = laser enable signal was not there

G = laser temp was high

H = laser temp was low

I = laser power was high

J = laser power was low

K = accelerometer was triggered

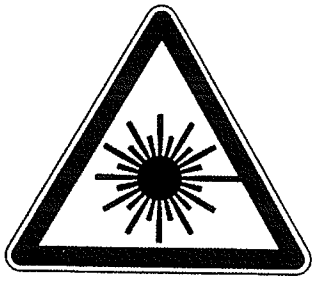
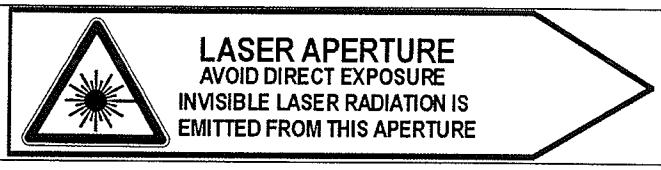
L = Lamp not OK

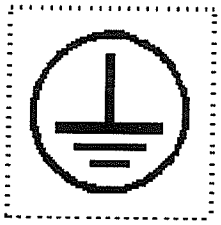
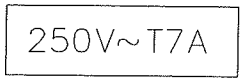
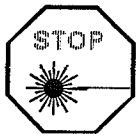
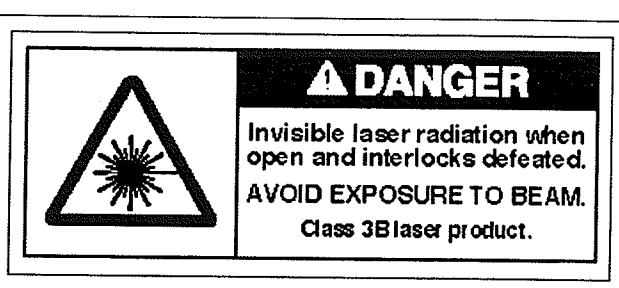
M = shutter closed

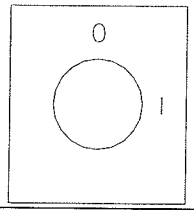
U = normal off

14. Labeling

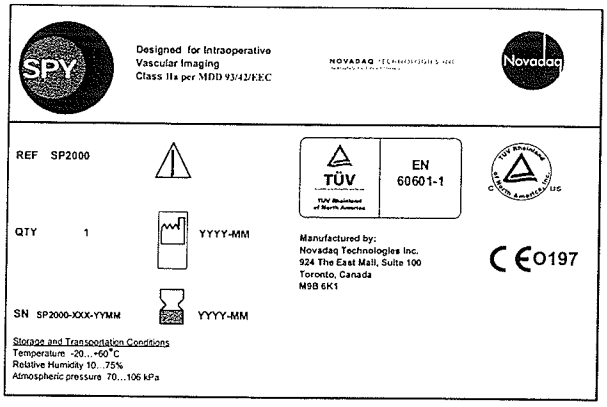
The following labels have been placed on the SPY™ system for operator and patient safety.

Label Name	Label	Location
1. Explanatory Label	INVISIBLE LASER RADIATION- AVOID EXPOSURE TO BEAM CLASS 3B LASER PRODUCT 3W MAXIMUM OUTPUT- 806 NM EN 60825 -1: 1994, IEC 60825 - 1 ED	On front door, left upper corner.
2. Caution Label		On front door, right front corner.
3. Laser Aperture	 LASER APERTURE AVOID DIRECT EXPOSURE INVISIBLE LASER RADIATION IS EMITTED FROM THIS APERTURE	On curved portion of head opposite the laser aperture.
4. Power, Input	INPUT POWER: 120V~, 350VA, 50/60HZ REPLACE WITH 8.0A/250V~ TIME LAG TYPE OR EQUIVALENT INPUT POWER: 100V~, 350VA, 50/60HZ REPLACE WITH 8.0A/250V~ TIME LAG TYPE OR EQUIVALENT INPUT POWER: 230V~, 350VA, 50/60HZ REPLACE WITH 4.0A/250V~ TIME LAG TYPE OR EQUIVALENT **Power, Input**	On electronic enclosure, right above power entry module
5. Power, Output	TOTAL MAXIMUM OUTPUT: 120V~, 5A MAX, 50/60HZ **Power, Output**	Electronic enclosure, right side of power connector.

Label Name	Label	Location
6. General Danger		1. On cart on the door that opens 2. On electronic enclosure front center
7. Electrical Danger		On electronic enclosure front center
8. Ground		1.On the outside (rear) of the electrical enclosure above the ground stud that accepts the ground wire that grounds the electrical enclosure to the cart ground strip 2. Adjacent to the cart ground strip located below the cart cable access grill at the back of the cart.
9. Fuse Labels	 	Applied directly on the top of each of the terminal block fuse holders.
10. Type CF Equipment		On head, on top, right of mechanical shutter
11. Laser Stop		On the red emergency button
12. Fiber Coupling		On top of the laser coupling so the arrow points to the mark on the coupling.
13. Laser Hazard		Placed on the bottom side of the imaging head.

Label Name	Label	Location
14. Laser Interlock Key Switch		Above Laser Interlock Key Switch
15. IR LED Switch	I IR LED ON O IR LED OFF	On the IR LED switch
16. System Power Switch	I System ON O System OFF	On the front of the Electrical Enclosure on the power switch.
17.Novadaq Service Personnel Only	**SPY SYSTEM TO BE SERVICED BY QUALIFIED NOVADAQ PERSONNEL ONLY** CONTACT DISTRIBUTOR FOR SERVICE: XXXXX XXXXXX XXXXXXX XXXXXXXX XXXXXXXXX XXXXXXXXXX XXXXXXXXXXX	On the back of the SPY™ unit.
18. Calibration Sticker	NEXT CALIBRATION DUE: _____/_____ Month Year LAST CALIBRATION: _____/_____ Month Year FOR CALIBRATION CONTACT DISTRIBUTOR: XXXXX XXXXXX XXXXXXX XXXXXXXX XXXXXXXXX XXXXXXXXXX XXXXXXXXXXX XXXXXXXXXXXX	On the inside door of the SPY™ unit. Record the calibration date and the next calibration date with non-erasable ink.

Novadaq Technologies™ SPY™ Intra-operative Imaging System

Label Name	Label	Location
19. SPY™ Label		On the back of the SPY™ System

Device Classification as per EN 60601-1:

Class I Equipment

Type CF Equipment

The device should not be used in a flammable atmosphere.

Suitable for continuous operation

Not protected from ingress of liquid

Power Rating: 350VA

MAINTENANCE AND CLEANING

The imaging head, arm and cart of the device should be cleaned during the regular cleaning of the equipment of the operating room. The non-optical system components shall be able to be cleaned with common hospital disinfecting agents, ex. END BAC 256, which contains n-alkyl dimethyl ammonium chloride (8.19%), didecyl dimethyl ammonium chloride (8.7%) and water (70-80%). We strongly recommend avoidance of any bleach-based cleaning agents. The preferred method of cleaning is a wipe-down with a damp cloth. We strongly recommend not spraying the device with the cleaning agent. Should the optical window require cleaning, we recommend the use of 70% alcohol.

NOTE: THE UNIT SHOULD BE UNPLUGGED FROM THE AC POWER BEFORE CLEANING TO REDUCE THE CHANCE OF ELECTRICAL SHOCK.

TROUBLESHOOTING THE NOVADAQ SPY™ SYSTEM

Initiation of Procedure

The SPY™ system is designed to be easy to use. Simply plug the system's power cord into an appropriately rated AC power outlet and turn the system POWER on. The system will be ready to use as outlined in the following steps.

- Turn KEY LOCK to the ON position.
- Position the "Imaging Head" approximately 12 inches (30cm) over the desired imaging area.
- Allow the "Imaging Head" position to stabilize (i.e. stop moving).

IF the system READY lamp does not illuminate, proceed to the troubleshoot section.

Problem:	Possible Cause:	Corrective Action:
READY light does not illuminate: Motion – red light	Imaging Head moving at the time of the assessment.	Keep head absolutely still and check READY light.
READY light does not illuminate: Focus – red light	Head not at proper distance.	Verify the distance indicator has center green LED illuminated. Move Head as required so the center green LED illuminates
READY light does not illuminate: System error message	System error	Turn unit off immediately. Call qualified service personnel.
Excessive force is required to manipulate imaging head	Wear of mechanical components of articulating arm	Call qualified service personnel.
Excessive force is required to roll device	Wheel lock is in place.	Release lock.
	Wear of mechanical components wheel mechanisms.	Call qualified service personnel.

If the problem does not fall within the scope of the table above, please call a qualified service technician.

CALIBRATING THE NOVADAQ SPY™ SYSTEM

Qualified service personnel will calibrate The Novadaq SPY™ system on a yearly basis. Novadaq will arrange for the calibration and will provide a minimum of 2 weeks notice before calibration is to take place.

Replacement of fuses

The required replacement fuse:
For 100V~ or 120V~ system: Time Delay, 8 Amp, 250V~, size: 5x20mm
For 230V~ system: Time Delay, 4 Amp, 250V~, size: 5x20mm.

Environmental conditions for transport and storage

Temperature –20 . . . +60C
Relative Humidity 10 . . . 75%
Atmospheric Pressure 70 . . . 106 kPa

CONTACT / SERVICE INFORMATION

For service please contact the distributor of the Novadaq SPY™ System in your country.

Warranty:

Novadaq Technologies warrants that its products substantially conform to Novadaq Technologies published specifications for such product for a period of (12) twelve months from the date of shipment and will replace products confirmed by Novadaq Technologies as defective products, at Novadaq Technologies' sole option, during said period. This warranty is contingent upon proper use of products in the application for which they were intended as indicated in the product label claims and Novadaq Technologies makes no warrant (express, implied or statutory) for products that are modified or subjected to unusual physical or electrical stress. With regard to Novadaq Technologies' products, which are labeled FOR SINGLE USE ONLY or DO NOT REUSE, this warranty is null and void following the multiple use of the products. Novadaq Technologies does not provide a warranty for those products subjected to resterilization and /or reuse unless the products are specifically designed and labeled for reuse.

EXCEPT FOR THE LIMITED WARRANTY PROVIDED ABOVE, NOVADAQ TECHNOLOGIES GRANTS NO OTHER WARRANTIES OR CONDITIONS, EXPRESS OR IMPLIED, BY STATUE IN ANY COMMUNICATIONS WITH BUYER, OR OTHERWISE, REGARDING THE PRODUCTS, THEIR FITNESS FOR ANY PURPOSE, THEIR QUALITY OF MERCHANTABILITY. NOVADAQ TECHNOLOGIES NIETHER ASSUMES, NOR AUTHORIZES ANY OTHER CONNECTION WITH THE SALE OR USE OF ANY PRODUCT. IN NO EVENT SHALL NOVADAQ TECHNOLOGIES BE LIABLE FOR THE PROCUREMENT OF SUBSTITUTED GOODS BY THE CUSTOMER OR FOR ANY CONSEQUENTIAL OR INCIDENTAL DAMAGES FOR BREACH OF WARRANTY.

APPENDICES

B: SYSTEM RISK ANALYSIS

1) INTRODUCTION

Purpose of this Document

This document assesses the potential hazards of the SPY™ System. The intent of the SRA is to anticipate such hazards so that they may be eliminated, reduced in severity, or reasonably controlled. The document will become an engineering design input. This document includes:

- Identified hazards
- List of potential causes for each hazard
- Severity ratings for each hazard
- Estimated probability of occurrence for each potential cause
- Minimum response or mitigation for each potential cause
- Method of implementation for mitigations
- Final Risk Index based on hazard severity and the probability of its causes

Device

The device is an SP-2000 infrared fluorescent imaging system that has been upgraded to include software and a computer system. The function of the software is purely for digital image acquisition and display of images. There is no capability to process or modify the images in any fashion. The laser illumination can be activated by software control however the laser is deactivated by the hardware timer set to 34 seconds.

Scope

The SRA identifies hazards associated with the SPY™ System including hazards caused by expected operator misuse. It does not cover hazards that may be caused by any equipment or accessories (except the dye and sterile drape) used in conjunction with the SPY™ System.

Definitions, Acronyms, and Abbreviations

False Negative:	There is a leak or occlusion that is not detected
False Positive:	A leak or occlusion is detected but does not exist
IC Green:	Indocyanine Green (the imaging agent). Also known as Cardio Green.
SRA:	System Risk Analysis (this document)

2) HAZARD ASSESSMENT METHOD

This System Risk Analysis uses a formalized approach to define, evaluate, and determine the proper hazard control requirements for the SPY™ System. The process used in this document to generate the System Risk Analysis is as follows:

1. All known hazards are listed.
2. Each listed hazard is rated for its severity. (Refer to Section 2.1. for severity codes.)
3. All known potential causes for each of the identified hazards are listed.
4. The minimum response required to control each cause of each hazard is then defined based on the risk index. (Refer to Section 2.4. for minimum response category definitions.)
5. The method of implementation for the minimum response is indicated.
6. Each identified cause for each hazard is rated for its probability of occurrence. (Refer to Section 2.2. for occurrence codes.) This is the probability of occurrence after the application of mitigation.
7. A risk index is determined for each cause of each hazard based on the lookup table in Section 2.3. This is the risk index after the application of mitigation.
8. All of the above information is tabulated. (Refer to the table in Section 3.)

Hazard Severity Ratings

The severity ratings apply only to the hazards and are not modified by the probability of occurrence or any mitigation efforts. The hazard severity ratings have the following meanings:

Severity Descriptor	**Definition**
Minor	Likely to result in a minor injury to the operator or the patient. A minor severity level is used to represent a nuisance such as a superficial cut which may require an adhesive bandage.
Moderate	Likely to result in a moderate injury to the operator or the patient. A moderate severity level represents conditions, which are medically treatable but require physician intervention such as a deep wound.

Major	Likely to result in death or a serious injury to the operator or the patient. Serious injuries refer to non-reversible injuries such as loss of limb.

Probability of Occurrence Ratings

For each of the potential causes of each hazard, an estimated probability of occurrence is listed. The probability of occurrence describes the probability that the potential cause will lead to the hazard. The probability of occurrence takes into account the hazard mitigation which will be applied. The levels of probability of occurrence and their meanings are listed below:

Occurrence	**Probability for Individual Item**	**Probability for Fleet or Inventory**
Improbable	Cannot be distinguished from zero	Assumed not to occur
Remote	Unlikely but possible	Occurrence is probable
Occasional	Likely to occur at least once	Will occur several times
Reasonable	Will occur several times	Will occur frequently
Frequent	Likely to occur frequently	Continuously experienced

Risk Index Ratings

The severity of the hazard and the probability of occurrence are used to determine a final risk index. This information is used during the design phase to determine which system failure modes need to be mitigated and which ones require special attention during validation. This ensures that all hazards have been acceptably mitigated.

The risk index codes are defined as follows:

Risk Index Code	Acceptance Criteria Description
U	Unacceptable, system redesign or program re-direction required.
N	Undesirable, decision required by Novadaq Technologies, Inc. senior management, with written notice to Design History File and the appropriate project team members.
R	Acceptance upon review by Quality Assurance with Novadaq Technologies, Inc. management approval.
A	Acceptable without review.

The following table shows the risk index codes for all possible combinations of Probability of Occurrence and Hazard Severity.

Probability of Occurrence	Hazard Severity		
	Minor	Moderate	Major
Improbable	A	A	R
Remote	A	R	R
Occasional	R	R	N
Reasonable	R	N	U
Frequent	N	U	U

Minimum Response Implementations

The implementation for the minimum response for each hazard or cause falls into one of the following categories:

Code	Response	Definition of minimum response
D	Design	The hazard will be mitigated by the actual design of the device.
L	Labeling/Training	The hazard will be mitigated by warning the operator about the hazard via a label or training. For example label on the instrument or instructions found in the user's manual for the safe handling of the device (training).
M	Maintenance	The hazard will be mitigated by requiring specific maintenance to the device.
P	Process (manufacturing)	The hazard will be mitigated by the manufacturing processes.
V	Validation	The hazard will be mitigated by design validation testing.

3) HAZARD

The following table represents the result of the System Risk Analysis for the SPY™ System.

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
False Positive	Inadequate illumination.	Moderate	Frequent	U	Distance Indicator.	Moderate	Remote	R
	Inadequate illumination.	Moderate	Frequent	U	Monitor illumination output through laser power indicator.	Moderate	Remote	R
	Inappropriate concentration or volume of dye.	Moderate	Frequent	U	Training/Labelling. Instructions in Operator's Manual	Moderate	Occasional	R
	Wrong timing.	Moderate	Reasonable	N	Training/Labelling. Instructions in Operator's Manual.	Moderate	Occasional	R
	Poor camera sensitivity.	Moderate	Occasional	R	System calibration with phantom.	Moderate	Improbable	A
	Camera focus.	Moderate	Frequent	U	Distance indicator.	Moderate	Remote	R
False Negative								
	Excess sensitivity.	Moderate	Occasional	R	System calibration with phantom.	Moderate	Improbable	A
	Over-illumination.	Moderate	Frequent	U	Control illumination output within accepted range through laser power indicator.	Moderate	Remote	R
	Inappropriate concentration or volume of dye.	Moderate	Frequent	U	Training/labelling. Instructions in Operator's Manual	Moderate	Occasional	R
	Laser power high	Moderate	Frequent	U	Laser power measurement by feedback to photodiode. Shut-off if laser power high.	Moderate	Remote	R
	Camera focus.	Moderate	Frequent	U	Distance indicator.	Moderate	Remote	R
No Observation	System failure.	Minor	Occasional	R	Training/labelling.	Minor	Occasional	R
Fire or explosion	Electrical short.	Major	Occasional	N	Design and test to meet regulatory standards.	Major	Improbable	R
	Electrical short.	Major	Occasional	N	Training/labelling.	Major	Improbable	R
	Overheated component.	Major	Occasional	N	Design and test to meet regulatory standards.	Major	Improbable	R
	Overheated component.	Major	Occasional	N	Training/labelling.	Major	Improbable	R

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
	Laser energy is concentrated on flammable surface due to component failure.	Major	Occasional	N	Device design - Loss of decollimation will interrupt feedback to photodiode. Will cut off laser power. Light guide securely fastened.	Major	Improbable	R
Electric shock to operator	Isolation failure.	Major	Reasonable	U	Design and test to meet regulatory standards.	Major	Improbable	R
	Electrical ground fault.	Major	Reasonable	U	Design and test to meet regulatory standards.	Major	Improbable	R
	Insulation failure.	Major	Reasonable	U	Design and test to meet regulatory standards.	Major	Improbable	R
	Electrical fault due to fluid ingress.	Major	Occasional	N	Design and test to meet regulatory standards. Training and labelling.	Major	Improbable	R
Electric shock to patient	Isolation failure.	Major	Reasonable	U	Design and test to meet regulatory standards.	Major	Improbable	R
	Electrical ground fault.	Major	Reasonable	U	Design and test to meet regulatory standards.	Major	Improbable	R
	Insulation failure.	Major	Reasonable	U	Design and test to meet regulatory standards.	Major	Improbable	R
	Electrical fault due to fluid ingress.	Major	Occasional	N	Design and test to meet regulatory standards. \ Training and labeling.	Major	Improbable	R
Burn or thermal injury to operator	Overheated component.	Moderate	Reasonable	N	Design and test to meet regulatory standards.	Moderate	Improbable	A
	Unintentional contact with concentrated laser emission due to component failure.	Moderate	Reasonable	N	Device design - Loss of decollimation will interrupt feedback to photodiode. Will cut off laser power. Light guide securely fastened.	Moderate	Improbable	A
	Excessive Laser Power	Major	Frequent	U	Laser power set within limits by feedback to photodiode. Power outside these limits will deactivate laser.	Major	Improbable	
	Laser does not turn off – Software malfunction	Major	Improbable	R	Software design - does not control laser activation or deactivation.			
	Laser power increase – software malfunction	Major	Improbable	R	Software design - does not control laser power.			
Burn or thermal injury to patient	Unintentional contact with concentrated laser emission due to component failure.	Moderate	Reasonable	N	Device design - Loss of decollimation will interrupt feedback to photodiode. Will cut off laser power. Light guide securely fastened.	Moderate	Improbable	A
	Excessive Laser Power	Major	Frequent	U	Laser power set within limits by feedback to photodiode. Power outside these limits will deactivate laser.	Major	Improbable	R

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
	Laser does not turn off – Software malfunction	Major	Improbable	R	Software Design – software does not control laser activation or deactivation			
	Laser power increase – software malfunction	Major	Improbable	R	Software Design – software does not control laser power			
Excess light injury to operator's eye	Operator error.	Major	Occasional	N	Training/labelling.	Major	Remote	R
	Excess Laser Output.	Major	Occasional	N	Laser power measurement by feedback to photodiode. Shut-off if laser power high.	Major	Remote	R
	Loss of collimation and resultant specular reflection of the beam.	Major	Occasional	N	Device design - Loss of decollimation will interrupt feedback to photodiode. Will cut off laser power. Securing of light guide.	Major	Remote	R
	Unintentional movement of Imaging Head.	Major	Occasional	N	Accelerometer and moving sensing circuit causes interruption of laser with movement. Mechanical Shutter is secondary mitigation if there is a failure of the accelerometer.	Major	Remote	R
	Incorrect aiming of Imaging Head	Major	Occasional	N	Range Detector, Training/Labelling	Major	Remote	R
Excess light injury to patient's eye	Operator error.	Major	Occasional	N	Training/labelling.	Major	Remote	R
	Excess Laser Output.	Major	Occasional	N	Laser power measurement by feedback to photodiode. Shut-off if laser power high.	Major	Remote	R
	Loss of colination and resultant specular reflection of the beam.	Major	Occasional	N	Device design - Loss of decollimation will interrupt feedback to photodiode. Will cut off laser power. Securing of light guide.	Major	Remote	R
	Unintentional movement of Imaging Head.	Major	Occasional	N	Accelerometer and moving sensing circuit causes interruption of laser with movement. Mechanical Shutter is secondary mitigation if there is a failure of the accelerometer.	Major	Remote	R
	Incorrect aiming of Imaging Head	Major	Occasional	N	Range Detector, Training/Labelling	Major	Remote	R
Interference with other devices	Device emits excessive EMI/RFI fields.	Major	Reasonable	U	Design and test to meet regulatory standards;	Major	Improbable	R

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
Device falls on operator	Operator error.	Moderate	Remote	R	Training/labelling.	Moderate	Improbable	A
	Improper center of gravity.	Moderate	Remote	R	Design and test to meet regulatory standards.	Moderate	Improbable	A
	Mechanical or structural failure of the device.	Moderate	Remote	R	Production Test and Adjustment.	Moderate	Improbable	A
Device falls on patient	Operator error.	Major	Remote	R	Training/labelling.	Major	Improbable	A
	Improper center of gravity.	Major	Remote	R	Design and test to meet regulatory standards.	Major	Improbable	A
	Mechanical or structural failure of the device.	Major	Remote	R	Production Test and Adjustment.	Major	Improbable	A
Abrasion, Cut or laceration injury to operator	Exposed sharp edges.	Minor	Occasional	R	Design to minimize exposed sharp edges.	Minor	Remote	A
	Exposed sharp edges.	Minor	Occasional	R	Training/labelling.	Minor	Remote	A
Operator allergic reaction	Operator error results in contact with dye.	Minor	Remote	A	Training/labelling.	Minor	Improbable	A
Patient allergic reaction	Clinician error (Failure to observe precautions).	Major	Remote	R	Training/labelling.	Major	Improbable	A
	Unexpected patient reaction.	Major	Remote	R	Training/labelling	Major	Remote	R
Over injection of dye	Operator error.	Minor	Reasonable	R	Training/labelling.	Minor	Improbable	A
Accidental injection of air	Operator error.	Major	Remote	R	Training/labelling.	Major	Improbable	A
Operator Infection	Operator error.	Moderate	Improbable	A	Training/labelling.	Moderate	Improbable	A
Patient Infection	Operator error.	Moderate	Remote	R	Sterile drape	Moderate	Remote	R
	Failure of drape.	Moderate	Reasonable	N	Design sterile drape to fit imaging head perfectly.	Moderate	Remote	R
	Non-sterile dye.	Moderate	Remote	R	Training/labelling. Instructions in Operator's Manual	Moderate	Improbable	A

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
	Non-Sterile Drape.	Moderate	Occasional	R	Training, Labelling, Sterilization Validation.	Moderate	Improbable	A
Repetitive motion injury to operator	Component failure or wear.	Minor	Occasional	R	Arm designed for 10 years of use.	Minor	Improbable	A
Sudden contact to operator	Operator error.	Minor	Occasional	R	Training/labelling.	Minor	Reasonable	R
	Articulating Arm Failure	Minor	Occasional	R	Arm is designed and rated (validated) for use in the OR.	Minor	Remote	A
	Wear in the cart wheels locking mechanism.	Minor	Remote	A	Training/labelling.	Minor	Remote	R
Sudden contact to patient	Operator error.	Moderate	Occasional	R	Training/labelling.	Moderate	Occasional	R
	Articulating Arm Failure	Major	Occasional	N	Arm is designed and rated (validated) for use in the OR.	Major	Remote	R
	Wear in the cartwheels locking mechanism.	Moderate	Remote	R	Training/labelling.	Moderate	Occasional	R
Pinch injury to operator	Operator error.	Minor	Occasional	R	Training/labelling.	Minor	Remote	A
	Improper design and improper labelling.	Minor	Occasional	R	Design and Training/labelling.	Minor	Remote	A
	Mechanical or structural failure.	Minor	Occasional	R	Arm rated for 10 years of use.	Minor	Remote	A
Inadequate Imaging	Laser Power too short – Due to Software Malfunction	Minor	Improbable	A	Software Design – software does not control laser activation or deactivation			
	Laser Power too weak – Due to Software Malfunction	Minor	Improbable	A	Software Design – software does not control laser power intensity			
	Depth Sensor Failure – Due to Software Malfunction	Major	Improbable	R	Software Design – software does not control depth sensor			
	Accelerometer Failure – Due to Software Malfunction	Major	Improbable	R	Software Design – software does not control accelerometer			
	No image Capture – due to software malfunction	Minor	Improbable	A	Software Design			
	Initialization/Setup: Frame set too high	Minor	Improbable	R	No risk, obvious to user			

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
Sub-Standard Patient Care	Initialization/Setup: Frame set too low	Minor	Improbable	R	No risk, obvious to user			
	Initialization/Setup: Frame not set	Minor	Improbable	A	No risk, obvious to user			
	Initialization/Setup: Interval too high	Minor	Improbable	R	No risk, obvious to user			
	Initialization/Setup: Interval too low	Minor	Improbable	R	No risk, obvious to user			
	Initialization/Setup: Interval not set	Minor	Improbable	A	No risk, obvious to user			
	Initialization/Setup: Safety Limit – Maximum laser illumination time – too long	Major	Improbable	R	Software Design - software does not control laser activation or deactivation			
	Initialization/Setup: Safety Limit – Maximum laser illumination time – too short	Minor	Improbable	A	Software Design - software does not control laser activation or deactivation			
	Initialization/Setup: Safety Limit – Maximum laser illumination time – not set	Minor	Improbable	A	Software Design - software does not control laser activation or deactivation			
	FloVision Acquire- Data entry: Creation of Patient Record – incorrect data entry – operator fault	Major	Occasional	N	Addition of pop-up window to ensure correct data entry	Major	Improbable	R
	FloVision Acquire- Data entry Creation of Patient Record – incorrect data entry – software fault	Major	Improbable	R	No risk – needs error in Windows			
	FloVision Acquire- Data entry: Creation of Patient Record – data storage in incorrect file – operator fault	Major	Occasional	N	Training and Labelling; addition of pop up window to remind user to check patient info.	Major	Remote	R
	FloVision Acquire- Data entry : Creation of Patient Record – data storage in incorrect file – software fault	Major	Improbable	R	Extremely low risk – needs error in Windows			

Deleted: MInor

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
	FloVision Acquire- Data entry : Creation of Patient Record – data storage not performed – operator fault	Minor	Remote	A	Software design – program will not proced until data entered			
	FloVision Acquire- Data entry : Creation of Patient Record – data storage not performed – software fault	Minor	Improbable	A	Extremely low risk – needs error in Windows			
	FloVision Acquire- Data entry : Retrieval of Existing Record – data not retrieved – software fault	Minor	Occasional	R	No risk – obvious to user			
	FloVision Acquire- Data entry : Retrieval of Existing Record – incorrect record retrieved – software fault	Major	Improbable	R	No risk – data is interior to file which is named (impossible cause)			
	FloVision Acquire- Data entry : Retrieval of Existing Record – incorrect record retrieved – operator fault	Major	Occasional	N	Training and Labelling addition of pop up window to remind user to check patient info.	Major	Improbable	R
	FloVision Acquire - Preview: Record Function - Laser does not turn on	Minor	Occasional	R	No hazard. Malfunction obvious to user by way of laser power meter.			
	FloVision Acquire - Record:: Record Function - Laser power is too low	Minor	Improbable	A	Software Design – software does not control laser power			
	FloVision Acquire - Record:: Record Function - Laser power is too high	Major	Improbable	R	Software Design – software does not control laser power			
	FloVision Acquire - Record: Record Function - Image capture not turned on	Minor	Improbable	A	No hazard, image not on screen, obvious to user			
	FloVision Acquire - Record: : Record Function - Laser does not turn off	Major	Improbable	R	No hazard, hardware controls laser shutoff			

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
	FloVision Acquire - Record: : Record Function - Laser turns off before time limit	Minor	Improbable	A	No hazard, less energy. Malfunction obvious to user from observation of monitor screen.			
	FloVision Acquire - Record: : Record Function - Laser does not turn off after time limit	Major	Improbable	R	Hardware override, not controlled by software			
	FloVision Acquire - Record: : Record Function – Timers do not reset	Minor	Improbable	A	Low Hazard, hardware time limit will be in effect.			
	FloVision Acquire - Record: Review Function – Program does not advance to Save screen	Minor	Improbable	A	No hazard, malfunction obvious to user			
	FloVision Acquire - Record:: Review Function – Program advances to the wrong screen	Minor	Improbable	A	No hazard, malfunction obvious to user			
	FloVision Acquire - Record: : Exit – The screen does not change	Minor	Improbable	A	No hazard, screen stays where you are, malfunction obvious to user.			
	FloVision Acquire - Record: : Exit– The wrong screen appears	Minor	Improbable	A	No hazard malfunction obvious to user			
	FloVision Acquire - Save :Instant replay functiuon. Sequence does not play	Minor	Improbable	A	No hazard malfunction obvious to user			
	FloVision Acquire - Save: Store Selected Subset– subset not stored	Minor	Improbable	A	No hazard, data lost, no misinterpretation of data possible			

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
	FloVision Acquire - Save: Store Selected Subset - Frame set not stored in it entirety	Major	Improbable	R	No hazard, low probability as it requires complete computer failure			
	FloVision Acquire - Save: Store Selected Subset – Frame set stored at wrong location/identity	Major	Improbable	R	No hazard, data already in directory with patient's name			
	FloVision Acquire - Save: Acquire Another Sequence Function – Failure	Minor	Improbable	A	No hazard, stays in same screen and therefore, malfunction obvious to user.			
	FloVision Acquire - Save: Acquire Another Sequence Function – Go to the wrong screen	Minor	Improbable	A	Software Design – very low likelihood. Malfunction obvious to user.			
	FloVision Acquire - Save:Main menu Function – No change in screen	Minor	Improbable	A	No hazard, stays in same screen and therefore, malfunction obvious to user.			
	FloVision Acquire - Save; Main menu function– Move to wrong screen	Minor	Improbable	A	Software Design – Low likelihood. Malfunction obvious to user.			
	FloVision Acquire - Save: Image Subset Function – Wrong beginning frame - too low	Minor	Improbable	A	Software Design – extremely low likelihood. Malfunction obvious to user and does not cause misinterpretation of images.			
	FloVision Acquire - Save: Image Subset Function – Wrong beginning frame – too high	Minor	Improbable	A	Software Design – extremely low likelihood. Malfunction obvious to user and does not cause misinterpretation of images.			
	FloVision Acquire - Save: Image Subset Function – Wrong end frame - too low	Minor	Improbable	A	Software Design – extremely low likelihood. Malfunction obvious to user and does not cause misinterpretation of images.			
	FloVision Acquire - Save: Image Subset Function – Wrong end frame – too high	Minor	Improbable	A	Software Design – extremely low likelihood. Malfunction obvious to user and does not cause misinterpretation of images.			
	FloVision Acquire - Save: Comment Field – no entry	Minor	Improbable	A	Software Design – extremely low likelihood			

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
	FloVision Acquire - Save: Comment Field – Storage of incorrect information	Major	Improbable	R	Software Design – extremely low likelihood			
	FloVision Acquire - Save: Comment Field – Storage into wrong location/identity	Major	Improbable	R	Software Design – extremely low likelihood			
	FloVision Acquire - Save: Print function; failure	Minor	Improbable	A	Software Design – extremely low likelihood			
	FloVision retrieve, Select: Patient Selection – Patient data does not highlight	Minor	Improbable	A	No hazard, user can't advance			
	FloVision retrieve, Select: Patient Selection – Wrong data file is accessed	Major	Improbable	R	Software Design – extremely low likelihood			
	FloVision retrieve, Select: Patient Selection – No data file is accessed	Minor	Improbable	A	No hazard, low probability as it requires complete computer failure			
	FloVision retrieve, View – Failure to advance to screen	Minor	Improbable	A	Low probability; requires Windows failure. No hazard, program stays static.			
	FloVision retrieve, View – Advances to the wrong screen	Minor	Improbable	A	Software Design: Extremely low likelihood of occurrence.			
	FloVision retrieve, View – No image is displayed	Minor	Improbable	A	Hazard nil as failure obvious to user.			
	FloVision retrieve, View – Wrong image sequence is displayed	Major	Improbable	R	Software Design: Extremely low likelihood.			
	FloVision retrieve, View – Display frame rate too high	Major	Improbable	R	Software Design: Extremely low likelihood.			
	FloVision retrieve, View – Display frame rate too low	Major	Improbable	R	Software Design: Extremely low likelihood.			
	FloVision retrieve, View – Display frame rate not set	Minor	Improbable	A	Software Design: Extremely low likelihood.			

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
	FloVision retrieve, View – Forward function – Fails	Major	Improbable	R	No hazard as obvious to user. Low likelihood as it requires Windows and image board failure			
	FloVision retrieve, View – Forward function – advances in the wrong direction	Major	Improbable	R	Software Design: Extremely low likelihood.			
	FloVision retrieve, View – Reverse function – Fails	Major	Improbable	R	No hazard as obvious to user. Low likelihood as it requires Windows and image board failure			
	FloVision retrieve, View – Reverse function – advances in the wrong direction	Major	Improbable	R	Software Design: Extremely low likelihood.			
	FloVision retrieve, View – Bounce – Fails	Minor	Improbable	A	No hazard as obvious to user. Low likelihood as it requires Windows and image board failure			
	FloVision retrieve, View – Bounce – Malfunctions – Forward and reverse only	Minor	Improbable	A	Software Design: Extremely low likelihood.			
	FloVision retrieve, View – Start Play - Fails	Minor	Improbable	A	No hazard as obvious to user. Low likelihood as it requires Windows and image board failure			
	FloVision retrieve, View – Create Movie – Fails	Minor	Improbable	A	No hazard as failure results in no movie creation		Improbable	A
	FloVision retrieve, View – Create Movie – Data is stored in the wrong location/identity	Major	Improbable	R	Software Design: Extremely low likelihood		Improbable	R
	FloVision retrieve, View – Comment Field – Comment not displayed	Minor	Improbable	A	Software Design: Extremely low likelihood			
	FloVision retrieve, View – Comment Field – Wrong comment pulled up	Major	Improbable	R	Software Design: Extremely low likelihood			
	FloVision retrieve, View – New Sequence – Fails to change screen	Minor	Improbable	A	No hazard as screen stays as is and failure is obvious to user.			
	FloVision retrieve, View – New Sequence – Moves to wrong screen	Minor	Improbable	A	Software Design: Extremely low likelihood			
	FloVision retrieve, View – Print - fails				Software Design: Extremely low likelihood		Improbable	A

Hazard	Potential Cause	Severity Pre	Probability Pre	Initial Risk	Mitigation or Reason for None	Severity - Post	Probability - Post	Final Risk
	FloVision retrieve Main menu – Fails/No function	Minor	Improbable	A	No hazard in failure. Obvious to user.			
	FloVision retrieve Main menu – Moves to wrong screen	Minor	Improbable	A	Software Design: Extremely low likelihood			
	FloVision Copy: Create CD/DVD – Fails/No function, no CD created	Minor	Improbable	A	No hazard, failure results in no CD created.			
	FloVision Copy: Create CD/DVD – Storage to wrong destination	Minor	Improbable	A	Low probability of occurrence - hardwired to CD drive			
	FloVision Copy: Create CD/DVD – Alteration of patient identity	Major	Improbable	R	Software Design: Extremely low likelihood.			
	FloVision Copy: Create CD/DVD – Alteration in patient data	Major	Improbable	R	Software Design-size of data stored compared to original;if they don't match, error message appears			
	FloVision Copy: Main menu– Fails/No Function	Minor	Improbable	A	Software Design: Extremely low likelihood.			
	FloVision Copy: Main menu – Moves to wrong screen	Minor	Improbable	A	Software Design: Extremely low likelihood.			
	FloVision Archive – No function	Minor	Improbable	A	No hazard, failure results in no DVD created.			
	FloVision Archive – Storage to wrong destination	Minor	Improbable	A	Hardwired to DVD drive			
	Flovision: Archive – Alteration in patient identity	Major	Improbable	R	Software Design: Extremely low likelihood.			
	FloVision: Archive – Alteration of data	Major	Improbable	R	Software Design-size of data stored compared to original;if they don't match, error message appears			
	FloVision Archive – Main menu No Function/Fails	Minor	Improbable	A	Software Design: Extremely low likelihood.			
	FloVision Archive – Main menu – Moves to wrong screen	Minor	Improbable	A	Software Design: Extremely low likelihood.			

APPENDICES

C: SAFETY DATA

	Time (seconds)							
Pig number	0	5	10	15	20	25	30	35
1	Temperature °C (Mean and SD)							
	36.12	36.14	36.20	36.24	36.23	36.22	36.24	36.25
	0.09	0.07	0.05	0.05	0.05	0.04	0.05	0.05
2								
	38.60	38.57	38.59	38.60	38.60	38.60	38.60	38.60
	0.00	0.05	0.03	0.00	0.00	0.00	0.00	0.00
3								
	38.65	38.61	38.51	38.62	38.64	38.65	38.65	38.63
	0.05	0.03	0.03	0.04	0.05	0.05	0.05	0.05
4								
	36.14	36.24	36.26	36.31	36.35	36.38	36.41	36.43
	0.10	0.11	0.05	0.10	0.08	0.10	0.07	0.07
5								
	37.40	37.47	37.50	37.49	37.54	37.55	37.58	37.62
	0.08	0.09	0.09	0.13	0.13	0.12	0.10	0.06
6								
	38.26	38.29	38.32	38.32	38.34	38.34	38.34	38.35
	0.08	0.11	0.10	0.10	0.11	0.11	0.11	0.10

Table 1. Myocardial Temperature During Fluorescence Image Acquisition

Myocardial temperature was recorded in 6 pigs during fluorescence image acquisition. Temperature was measured at 5 second intervals throughout each imaging session. The imaging protocol was performed 10 times in each animal and the data for each time point averaged over the 10 imaging sessions. The data are reported as mean and standard deviation.

□□□

		Pig #1			Pig #2			Pig #3			Pig #4			Pig #5			Pig #6		
		HR (bpm)	PR (ms)	(SD) (ms)	HR (bpm)	PR (ms)	(SD) (ms)	HR (bpm)	PR (ms)	(SD) (ms)	HR (bpm)	PR (ms)	(SD) (ms)	HR (bpm)	PR (ms)	(SD) (ms)	HR (bpm)	PR (ms)	(SD) (ms)
Baseline	2 mins	108	97	(5.8)	105	125	(5.0)	87	115	(5.0)	93	110	(0.0)	99	110	(0.0)	120	100	(0.0)
	4 mins	108	100	(0.0)	105	110	(0.0)	87	118	(2.9)	93	110	(0.0)	99	110	(0.0)	120	98	(2.9)
	6 mins	108	100	(0.0)	108	125	(5.0)	84	117	(5.8)	93	110	(0.0)	99	110	(0.0)	120	98	(2.9)
	8 mins	108	100	(0.0)	105	110	(0.0)	84	117	(11.5)	93	110	(0.0)	99	107	(5.8)	120	98	(2.9)
	10 mins	108	100	(0.0)	105	130	(0.0)	84	120	(0.0)	93	110	(0.0)	99	108	(2.9)	120	97	(2.9)
	12 mins	111	103	(2.9)	105	123	(5.8)	84	118	(2.9)	93	110	(0.0)	101	107	(5.8)	117	98	(2.9)
	14 mins	114	102	(2.9)	105	125	(5.0)	84	113	(2.9)	96	110	(0.0)	99	105	(5.0)	117	98	(2.9)
	16 mins	114	100	(0.0)	108	127	(2.9)	87	110	(0.0)	96	107	(2.9)	102	107	(5.8)	117	97	(2.9)
	18 mins	114	102	(2.9)	108	107	(5.8)	90	117	(7.6)	96	110	(0.0)	102	107	(2.9)	117	98	(2.9)
	20 mins	114	103	(5.8)	108	110	(10.0)	87	125	(8.7)	96	110	(0.0)	102	107	(2.9)	117	97	(2.9)
	22 mins	114	102	(2.9)	108	115	(5.0)	96	118	(2.9)	96	110	(0.0)	102	105	(5.0)	117	97	(5.8)
	24 mins	114	102	(2.9)	108	107	(2.9)	102	120	(0.0)	93	110	(0.0)	102	108	(2.9)	120	95	(5.0)
	26 mins	120	105	(0.0)	108	112	(7.6)	102	120	(0.0)	93	110	(0.0)	102	105	(5.0)	120	95	(5.0)
	28 mins	120	103	(5.8)	110	120	(0.0)	102	122	(2.9)	93	110	(0.0)	102	105	(5.0)	120	95	(5.0)
	30 mins	120	102	(2.9)	114	112	(2.9)	102	123	(11.5)	96	110	(0.0)	102	105	(5.0)	120	92	(2.9)
Imaging Session	1	120	100	(0.0)	114	99	(2.2)	102	118	(4.5)	93	110	(0.0)	102	104	(5.5)	120	98	(4.5)
	2	120	101	(2.2)	114	101	(2.2)	102	122	(4.5)	90	110	(0.0)	105	109	(2.2)	120	99	(2.2)
	3	120	100	(0.0)	114	105	(5.0)	102	122	(4.5)	93	108	(2.7)	105	105	(5.0)	117	98	(2.7)
	4	120	101	(2.2)	111	100	(0.0)	102	121	(10.2)	93	109	(2.2)	105	105	(5.0)	120	98	(4.5)
	5	114	100	(0.0)	114	104	(5.5)	108	114	(5.5)	93	109	(2.2)	105	107	(4.5)	117	97	(4.5)
	6	117	100	(0.0)	111	100	(0.0)	108	114	(5.5)	96	108	(2.7)	105	107	(4.5)	120	96	(4.2)
	7	117	100	(0.0)	111	100	(0.0)	108	111	(8.9)	93	110	(0.0)	105	109	(2.2)	120	100	(0.0)
	8	117	100	(0.0)	111	100	(0.0)	108	108	(4.5)	93	107	(4.5)	105	105	(5.0)	117	99	(2.2)
	9	117	100	(0.0)	108	101	(2.2)	108	111	(4.2)	96	109	(2.2)	105	107	(4.5)	117	100	(0.0)
	10	117	100	(0.0)	108	100	(0.0)	108	111	(2.2)	96	105	(3.5)	105	110	(0.0)	120	100	(0.0)

		Pig #1			Pig #2			Pig #3			Pig #4			Pig #5			Pig #6		
		HR (bpm)	PR (ms)	(SD) (ms)	HR (bpm)	PR (ms)	(SD) (ms)	HR (bpm)	PR (ms)	(SD) (ms)	HR (bpm)	PR (ms)	(SD) (ms)	Hr (bpm)	PR (ms)	(SD) (ms)	HR (bpm)	PR (ms)	(SD) (ms)
Post Imaging	2 mins	117	100	(0.0)	111	100	(0.0)	108	112	(2.9)	99	110	(0.0)	105	108	(2.9)	120	97	(5.8)
	4 mins	114	100	(0.0)	111	100	(0.0)	108	113	(5.8)	99	110	(0.0)	105	103	(2.9)	117	98	(2.9)
	6 mins	114	100	(0.0)	111	100	(0.0)	108	115	(5.0)	99	107	(2.9)	105	108	(2.9)	117	100	(0.0)
	8 mins	111	100	(0.0)	111	100	(0.0)	108	117	(5.8)	96	110	(0.0)	105	108	(2.9)	117	100	(0.0)
	10 mins	114	100	(0.0)	108	100	(0.0)	108	107	(2.9)	96	110	(0.0)	105	110	(0.0)	114	100	(0.0)
	12 mins	114	100	(0.0)	108	98	(2.9)	108	113	(5.8)	96	110	(0.0)	105	108	(2.9)	114	97	(2.9)
	14 mins	111	100	(0.0)	111	100	(0.0)	108	108	(2.9)	96	107	(2.9)	105	110	(0.0)	117	100	(0.0)
	16 mins	108	100	(0.0)	111	100	(0.0)	108	112	(7.6)	96	110	(0.0)	105	110	(0.0)	117	98	(2.9)
	18 mins	108	100	(0.0)	114	100	(0.0)	111	112	(7.6)	96	108	(2.9)	105	110	(0.0)	114	100	(0.0)
	20 mins	108	100	(0.0)	108	103	(2.9)	108	120	(0.0)	96	107	(2.9)	105	108	(2.9)	117	100	(0.0)
	22 mins	108	100	(0.0)	108	100	(0.0)	111	110	(0.0)	99	107	(5.8)	105	107	(2.9)	114	100	(0.0)
	24 mins	105	100	(0.0)	111	100	(0.0)	111	113	(2.9)	96	110	(0.0)	108	110	(0.0)	114	100	(0.0)
	26 mins	102	100	(0.0)	111	100	(0.0)	111	113	(5.8)	96	105	(5.0)	108	110	(0.0)	114	100	(0.0)
	28 mins	102	100	(0.0)	111	100	(0.0)	111	113	(5.8)	102	107	(5.8)	108	110	(0.0)	114	100	(0.0)
	30 mins	105	100	(0.0)	114	100	(0.0)	108	113	(2.9)	102	107	(2.9)	108	108	(2.9)	114	100	(0.0)

Table 2. Effect of Fluorescence Imaging on PR Interval.
Electrocardiograms were collected from 6 pigs during a 30 min baseline period, 10 fluorescence image acquisitions and for a further 30 minutes following the final image acquisition. PR intervals were measured from hard copy traces of the ECG. Each data point is the mean derived from analysis of 5 cardiac cycles. HR, heart rate in beats per minute; PR, PR interval in ms; SD, standard deviation in ms.

		Pig #1			Pig #2			Pig #3			Pig #4			Pig #5			Pig #6		
		HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)
Baseline	2 mins	108	83	(5.8)	105	87	(5.8)	87	45	(5.0)	93	40	(0.0)	99	35	(5.0)	120	42	(2.9)
	4 mins	108	73	(5.8)	105	83	(5.8)	87	48	(2.9)	93	38	(2.9)	99	40	(0.0)	120	40	(0.0)
	6 mins	108	78	(2.9)	108	90	(0.0)	84	50	(0.0)	93	40	(0.0)	99	40	(0.0)	120	40	(0.0)
	8 mins	108	75	(5.0)	105	90	(0.0)	84	48	(2.9)	93	40	(0.0)	99	35	(5.0)	120	40	(0.0)
	10 mins	108	73	(2.9)	105	90	(0.0)	84	48	(2.9)	93	40	(0.0)	99	38	(2.9)	120	40	(0.0)
	12 mins	111	72	(2.9)	105	83	(5.8)	84	50	(0.0)	93	40	(0.0)	101	35	(5.0)	117	40	(0.0)
	14 mins	114	75	(5.0)	105	90	(0.0)	84	48	(2.9)	96	40	(0.0)	99	35	(5.0)	117	40	(0.0)
	16 mins	114	75	(0.0)	108	83	(5.8)	87	47	(5.8)	96	40	(0.0)	102	37	(5.8)	117	43	(5.8)
	18 mins	114	72	(2.9)	108	88	(2.9)	90	48	(2.9)	96	37	(5.8)	102	35	(5.0)	117	42	(2.9)
	20 mins	114	75	(5.0)	108	90	(0.0)	87	50	(0.0)	96	42	(7.6)	102	37	(5.8)	117	43	(5.8)
	22 mins	114	72	(2.9)	108	90	(0.0)	96	50	(0.0)	96	40	(0.0)	102	35	(5.0)	117	40	(0.0)
	24 mins	114	73	(5.8)	108	88	(2.9)	102	50	(0.0)	93	40	(0.0)	102	37	(5.8)	120	43	(2.9)
	26 mins	120	72	(2.9)	108	87	(5.8)	102	50	(0.0)	93	40	(0.0)	102	32	(2.9)	120	47	(2.9)
	28 mins	120	77	(2.9)	110	88	(2.9)	102	53	(5.8)	93	40	(0.0)	102	33	(5.8)	120	42	(2.9)
	30 mins	120	75	(5.0)	114	88	(2.9)	102	50	(0.0)	96	40	(0.0)	102	35	(5.0)	120	42	(2.9)
Imaging Sessions	1	120	75	(5.0)	114	89	(5.5)	102	51	(2.2)	93	39	(2.2)	102	39	(2.2)	120	44	(4.2)
	2	120	71	(2.2)	114	89	(2.2)	102	51	(2.2)	90	40	(0.0)	105	36	(5.5)	120	46	(4.2)
	3	120	74	(4.2)	114	89	(2.2)	102	52	(2.7)	93	40	(0.0)	105	36	(5.5)	117	46	(4.2)
	4	120	75	(3.5)	111	90	(0.0)	102	51	(2.2)	93	38	(4.5)	105	37	(4.5)	120	46	(4.2)
	5	114	70	(0.0)	114	88	(2.7)	108	51	(2.2)	93	39	(2.2)	105	41	(2.2)	117	44	(4.2)
	6	117	72	(2.7)	111	86	(4.2)	108	53	(4.5)	96	40	(0.0)	105	38	(4.5)	120	44	(4.2)
	7	117	72	(2.7)	111	85	(5.0)	108	53	(4.5)	93	38	(2.7)	105	33	(4.5)	120	43	(4.5)
	8	117	73	(2.7)	111	81	(2.2)	108	51	(2.2)	93	40	(0.0)	105	38	(4.5)	117	42	(4.5)
	9	117	75	(5.0)	108	79	(5.5)	108	51	(2.2)	96	40	(0.0)	105	40	(0.0)	117	45	(5.0)
	10	117	72	(2.7)	108	85	(3.5)	108	52	(2.7)	96	40	(0.0)	105	36	(5.5)	120	43	(4.5)

		Pig #1			Pig #2			Pig #3			Pig #4			Pig #5			Pig #6		
		HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)	HR (bpm)	QRS (ms)	(SD) (ms)
Post Imaging	2 mins	117	72	(2.9)	111	80	(0.0)	108	53	(2.9)	99	40	(0.0)	105	38	(2.9)	120	43	(5.8)
	4 mins	114	72	(2.9)	111	80	(0.0)	108	50	(0.0)	99	40	(0.0)	105	40	(0.0)	117	43	(2.9)
	6 mins	114	75	(5.0)	111	80	(0.0)	108	53	(5.8)	99	40	(0.0)	105	40	(0.0)	117	45	(5.0)
	8 mins	111	72	(2.9)	111	78	(2.9)	108	50	(0.0)	96	40	(0.0)	105	40	(0.0)	117	42	(2.9)
	10 mins	114	75	(5.0)	108	80	(0.0)	108	50	(0.0)	96	40	(0.0)	105	40	(0.0)	114	40	(0.0)
	12 mins	114	73	(5.8)	108	80	(0.0)	108	50	(0.0)	96	40	(0.0)	105	38	(2.9)	114	42	(2.9)
	14 mins	111	70	(0.0)	111	82	(2.9)	108	52	(2.9)	96	40	(0.0)	105	40	(0.0)	117	45	(5.0)
	16 mins	108	75	(5.0)	111	82	(2.9)	108	57	(5.8)	96	40	(0.0)	105	38	(2.9)	117	42	(2.9)
	18 mins	108	72	(2.9)	114	80	(0.0)	111	50	(0.0)	96	40	(0.0)	105	38	(2.9)	114	42	(2.9)
	20 mins	108	70	(0.0)	108	80	(0.0)	108	50	(0.0)	96	40	(0.0)	105	38	(2.9)	117	43	(2.9)
	22 mins	108	75	(5.0)	108	80	(0.0)	111	52	(2.9)	99	40	(0.0)	105	37	(5.8)	114	42	(2.9)
	24 mins	105	72	(2.9)	111	80	(0.0)	111	50	(0.0)	96	40	(0.0)	108	37	(5.8)	114	47	(2.9)
	26 mins	102	72	(2.9)	111	80	(0.0)	111	50	(0.0)	96	40	(0.0)	108	35	(5.0)	114	42	(2.9)
	28 mins	102	73	(5.8)	111	82	(2.9)	111	50	(0.0)	102	37	(2.9)	108	35	(5.0)	114	45	(5.0)
	30 mins	105	70	(0.0)	114	80	(0.0)	108	50	(0.0)	102	38	(2.9)	108	37	(2.9)	114	42	(2.9)

Table 3. Effect of Fluorescence Imaging on QRS Interval.
Electrocardiograms were collected from 6 pigs during a 30 min baseline period, 10 fluorescence image acquisitions and for a further 30 minutes following the final image acquisition. QRS intervals were measured from hard copy traces of the ECG. Each data point is the mean derived from analysis of 5 cardiac cycles. HR, heart rate in beats per minute; QRS, QRS interval in ms; SD, standard deviation in ms.

		Pig #1			Pig #2			Pig #3			Pig #4			Pig #5			Pig #6		
		HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)
Baseline	2 mins	108	387	(11.5)	105	307	(5.8)	87	325	(5.0)	93	417	(25.2)	99	348	(10.4)	120	340	(10.0)
	4 mins	108	375	(5.0)	105	310	(0.0)	87	333	(5.8)	93	427	(20.8)	99	340	(0.0)	120	335	(8.7)
	6 mins	108	380	(10.0)	108	305	(5.0)	84	333	(5.8)	93	433	(28.9)	99	340	(10.0)	120	338	(2.9)
	8 mins	108	360	(15.0)	105	303	(5.8)	84	332	(2.9)	93	413	(32.1)	99	342	(2.9)	120	335	(5.0)
	10 mins	108	375	(13.2)	105	300	(0.0)	84	332	(7.6)	93	402	(7.6)	99	340	(0.0)	120	338	(18.9)
	12 mins	111	368	(18.9)	105	302	(2.9)	84	332	(2.9)	93	405	(13.2)	101	337	(5.8)	117	337	(5.8)
	14 mins	114	355	(17.3)	105	307	(2.9)	84	330	(0.0)	96	393	(5.8)	99	333	(5.8)	117	333	(15.3)
	16 mins	114	355	(8.7)	108	303	(2.9)	87	323	(5.8)	96	403	(11.5)	102	337	(5.8)	117	337	(15.3)
	18 mins	114	345	(5.0)	108	303	(2.9)	90	323	(5.8)	96	410	(10.0)	102	337	(11.5)	117	340	(0.0)
	20 mins	114	352	(2.9)	108	302	(2.9)	87	320	(0.0)	96	393	(11.5)	102	338	(7.6)	117	353	(5.8)
	22 mins	114	347	(5.8)	108	302	(2.9)	96	323	(5.8)	96	393	(15.3)	102	347	(5.8)	117	338	(7.6)
	24 mins	114	348	(7.6)	108	300	(0.0)	102	320	(10.0)	93	412	(5.8)	102	340	(10.0)	120	337	(5.8)
	26 mins	120	345	(8.7)	108	300	(0.0)	102	308	(2.9)	93	423	(5.8)	102	337	(5.8)	120	337	(5.8)
	28 mins	120	342	(7.6)	110	298	(2.9)	102	322	(7.6)	93	417	(23.1)	102	343	(5.8)	120	333	(5.8)
	30 mins	120	343	(5.8)	114	298	(2.9)	102	322	(2.9)	96	408	(10.4)	102	340	(10.0)	120	333	(5.8)
Imaging Sessions	1	120	345	(3.5)	114	299	(2.2)	102	327	(4.5)	93	429	(21.9)	102	344	(8.9)	120	335	(5.0)
	2	120	333	(4.5)	114	304	(6.5)	102	328	(4.5)	90	431	(19.5)	105	348	(4.5)	120	337	(8.4)
	3	120	342	(8.4)	114	301	(4.2)	102	325	(5.0)	93	412	(8.4)	105	340	(0.0)	117	347	(14.0)
	4	120	345	(8.7)	111	299	(2.2)	102	326	(4.2)	93	423	(22.2)	105	346	(11.4)	120	340	(7.1)
	5	114	344	(5.5)	114	301	(2.2)	108	321	(5.5)	93	430	(15.8)	105	343	(6.7)	117	334	(8.9)
	6	117	349	(8.9)	111	304	(5.5)	108	322	(4.5)	96	418	(13.5)	105	333	(6.7)	120	336	(5.5)
	7	117	351	(7.4)	111	300	(0.0)	108	319	(5.5)	93	429	(24.1)	105	331	(2.2)	120	334	(11.4)
	8	117	346	(5.5)	111	299	(2.2)	108	319	(5.5)	93	409	(11.4)	105	345	(15.8)	117	341	(14.3)
	9	117	354	(8.9)	108	299	(2.2)	108	318	(4.5)	96	414	(11.9)	105	346	(15.2)	117	340	(7.1)
	10	117	349	(7.4)	108	299	(2.2)	108	322	(4.5)	96	421	(18.8)	105	354	(13.4)	120	338	(4.5)

		Pig #1			Pig #2			Pig #3			Pig #4			Pig #5			Pig #6		
		HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)	HR (bpm)	QT (ms)	(SD) (ms)
Post Imaging	2 mins	117	350	(5.0)	111	298	(2.9)	108	323	(5.8)	99	407	(20.8)	105	340	(10.0)	120	353	(11.5)
	4 mins	114	355	(5.0)	111	297	(5.8)	108	322	(10.4)	99	430	(26.5)	105	340	(10.0)	117	347	(15.3)
	6 mins	114	355	(8.7)	111	300	(0.0)	108	320	(0.0)	99	397	(11.5)	105	350	(17.3)	117	340	(5.0)
	8 mins	111	352	(7.6)	111	302	(2.9)	108	322	(2.9)	96	425	(13.2)	105	337	(15.3)	117	357	(15.3)
	10 mins	114	362	(7.6)	108	298	(2.9)	108	322	(2.9)	96	467	(5.8)	105	330	(0.0)	114	337	(5.8)
	12 mins	114	367	(10.4)	108	297	(5.8)	108	320	(0.0)	96	423	(20.8)	105	343	(5.8)	114	347	(5.8)
	14 mins	111	363	(5.8)	111	300	(0.0)	108	323	(5.8)	96	422	(33.3)	105	333	(5.8)	117	350	(10.0)
	16 mins	108	363	(5.8)	111	297	(5.8)	108	320	(0.0)	96	402	(10.4)	105	335	(8.7)	117	353	(5.8)
	18 mins	108	382	(7.6)	114	298	(2.9)	111	317	(5.8)	96	417	(20.8)	105	330	(10.0)	114	343	(11.5)
	20 mins	108	373	(5.8)	108	302	(2.9)	108	317	(2.9)	96	393	(2.9)	105	340	(17.3)	117	347	(5.8)
	22 mins	108	382	(10.4)	108	298	(2.9)	111	318	(2.9)	99	407	(28.9)	105	333	(15.3)	114	353	(5.8)
	24 mins	105	380	(10.0)	111	298	(7.6)	111	317	(5.8)	96	427	(32.1)	108	330	(10.0)	114	347	(11.5)
	26 mins	102	380	(0.0)	111	297	(5.8)	111	313	(5.8)	96	427	(35.1)	108	323	(5.8)	114	357	(5.8)
	28 mins	102	397	(15.3)	111	295	(5.0)	111	315	(5.0)	102	430	(10.0)	108	323	(5.8)	114	347	(5.8)
	30 mins	105	383	(23.6)	114	298	(2.9)	108	312	(2.9)	102	377	(5.8)	108	337	(2.9)	114	357	(5.8)

Table 4. Effect of Fluorescence Imaging on QT Interval.
Electrocardiograms were collected from 6 pigs during a 30 min baseline period, 10 fluorescence image acquisitions and for a further 30 minutes following the final image acquisition. QT intervals were measured from hard copy traces of the ECG. Each data point is the mean derived from analysis of 5 cardiac cycles. HR, heart rate in beats per minute; QT, QT interval in ms; SD, standard deviation in ms.

	Pig #1			Pig #4			Pig #5			Pig #6		
	Heart Rate (bpm)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Heart Rate (bpm)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Heart Rate (bpm)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Heart Rate (bpm)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)
Pre imaging 1	120	94	64	N/A	N/A	N/A	N/A	92	58	117	94	63
Imaging 1	120	94	64	N/A	N/A	N/A	105	93	59	117	95	63
Pre imaging 2	120	97	65	90	104	90	105	97	62	117	97	65
Imaging 2	120	95	65	90	104	90	102	96	60	117	96	65
Pre imaging 3	N/A	95	64	93	102	90	102	96	61	117	96	65
Imaging 3	120	94	63	93	102	92	102	94	60	117	97	65
Pre imaging 4	117	98	65	93	98	89	102	91	57	117	100	68
Imaging 4	120	98	65	93	98	89	102	90	58	117	97	66
Pre imaging 5	114	96	64	96	98	88	105	91	58	117	97	65
Imaging 5	117	97	64	96	98	88	102	89	56	117	97	65
Pre imaging 6	114	96	63	96	100	88	102	90	58	117	95	63
Imaging 6	117	94	62	96	100	88	105	94	60	117	97	65
Pre imaging 7	114	96	63	96	101	86	105	91	59	120	95	64
Imaging 7	117	99	64	96	102	87	102	91	58	120	96	64
Pre imaging 8	114	97	64	93	102	86	105	91	59	117	95	64
Imaging 8	117	97	65	93	102	86	105	92	59	120	95	63
Pre imaging 9	114	97	64	96	102	86	105	90	57	117	95	63
Imaging 9	114	96	64	96	102	86	105	89	57	120	95	63
Pre imaging 10	111	96	63	96	101	85	102	90	58	120	94	62
Imaging 10	114	96	63	96	102	87	105	93	59	117	93	60

Table 5. Effect of Fluorescence Imaging on Arterial Pressure
Arterial pressures were measured in the carotid arteries of 4 pigs immediately before, and during 10 consecutive image sequence acquisitions

	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6
	Creatine Kinase Activity (Units/L)					
Baseline	1045 1053	356 347	227 225	424 419	736 741	1068 1073
Post Imaging	1109 1098	447 450	254 257	434 438	785 791	1018 1022

Table 6. Effect of Fluorescence Imaging on Creatine Kinase Release.
Creatine kinase levels were determined in blood samples taken immediately prior to starting the first image sequence acquisition (Baseline) and upon completion of the 30 minute ECG data acquisition period following the final image sequence acquisition (Post Imaging). The results are presented as duplicate determinations.