SAFETY AND FEASIBILITY OF ACTIVSIGHT™ LASER SPECKLE IMAGING IN VISUALIZATION OF TISSUE PERFUSION AND CRITICAL STURCTURES IN HUMAN

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PRECIS

Background:

- The integrity of vasculature and tissue perfusion at the operative site remains a critical local determinant in expectant tissue healing, avoiding complications and ensuring good clinical outcome. Today, the most commonly used method of assessing vascularity and tissue perfusion intraoperatively however continues to be a simple visual inspection of tissue by naked human eyes guided by recall and experience regardless of surgical approach, MIS or open.
- The currently available additional means of visualizing blood flow intraoperatively are limited to radiation-based fluoroscopy with X-ray contrast dye or contrast agent such as a fluorophore-based near infrared camera system. These approaches are suboptimal in effectiveness (non-real time, lacks objectivity and specificity), cumbersome to efficient workflow (need for contrast reagent preparation and injection, potential adverse allergic reactions, risk of radiation exposure and/or training requirement), and efficacious utility (need for large capital equipment, space and cost).
- Over 4 million soft tissue anastomoses are performed without precise knowledge of perfusion and vascular status of the tissue today. Accurate, real-time visualization of blood flow and tissue perfusion would have critical impact on clinical outcome and reducing complications.
- An optical imaging based on monochromatic coherent light known as Laser-speckle-contrast imaging (LSCI) represents a label-free imaging method using coherent monochromatic light where blood flow and tissue perfusion can be detected. Dye-free, intraoperative visualization of critical hidden, sub-surface structures in real-time obviate the above disadvantages of dye-based visualization and would be ideal most surgical procedures.
- **ActivSight™** combines an innovative form factor and proprietary software to deliver precise, objective, cost efficient real-time visualization of blood flow and tissue perfusion intraoperatively for laparoscope-based surgery. A small adaptor that fits between any existing laparoscope and camera systems and a separate light source placed along any current commercial system will deliver objective real-time tissue perfusion and blood flow information intraoperatively.
- Interestingly, our recent preclinical data also indicate that **ActivSight™** can also readily display an ICG-based visualization of the biliary tree in real time at equivalent or superior to current commercial products. The innovative form factor of **ActivSight™** enables any laparoscopic system for ICG-based visualization at a fraction of the cost of current competitor with minimal disruption to workflow.
- Bile duct injury (BDI) during cholecystectomy is a serious surgical complication with a significant risk of early death, serious ongoing morbidities including multiple reinterventions requiring prolonged and repeated hospital stay, over a billion dollars in healthcare costs alone in US alone each year. The introduction laparoscopic approach despite several benefits, however, resulted in increased incidence of BDI up to 4-folds in some reports.
- Most major biliary injuries result from misidentification, either misidentification of the common bile duct as duct as the cystic duct or misidentification of an aberrant bile duct. It is increasingly clear that the use of intraoperative cholangiography (IOC) has a significant association with improved survival. The recent 'state of the art consensus conference on prevention of bile duct injury during cholecystectomy' in 2018 strongly recommends that the use of intraoperative biliary imaging to mitigate the risk of BDI during laparoscopic cholecystectomy (LC) in patients with uncertain anatomy or suspicion of BDI and the liberal use of IOC during any LC. Performing IOC laparoscopically however challenges surgeons' skills, is time-consuming, and requires a learning curve to interpret images.
- The currently available additional means of visualizing biliary tree intraoperatively are limited to radiation-based fluoroscopy with X-ray contrast dye. These approaches are suboptimal in effectiveness

(non-real time, lacks objectivity and specificity), cumbersome to efficient workflow (need for contrast reagent preparation and injection, potential adverse allergic reactions, risk of radiation exposure and/or training requirement), and efficacious utility (need for large capital equipment, space and cost).

- Recent randomized controlled trial, near-infrared fluorescent cholangiography (NIFC) was statistically superior to white light (WLI) alone visualizing extrahepatic biliary structures during laparoscopic Cholecystectomy. Pre-dissection detection rates were significantly superior in the NIFC group for all 7 biliary structures. After dissection, similar intergroup differences were observed for all structures except CD and CGJ, for which no differences were observed. Increased body mass index was associated with reduced detection of most structures in both groups, especially before dissection. Only 2 patients, both in the WL group, sustained a biliary duct injury.

ActivSight™ poses non-significant low safety concerns based on the fact that the device has no direct patient contact, and that there are a number of existing Near Infrared (NIR) based predicate devices already in clinical practice. The proposed human trial poses a minimal risk and maximum potential benefits for many clinical unmet needs. Activ Surgical herein proposes a safety and feasibility trial.

Primary Objective: To determine safety and feasibility of **ActivSight™** in displaying tissue perfusion in intestinal anastomoses including colorectal and bariatric surgery.

Secondary Objective: To determine the efficacy of **ActivSight™** in; (1) displaying tissue vascularity and perfusion in comparison to ICG during gastrointestinal anastomoses; and (2) displaying biliary tree during laparoscopic cholecystectomy using ICG-based IOC.

The clinical metrics for secondary objectives on tissue perfusion and vascularity include preparation time (single versus multiple injections of ICG), latency of display (real-time versus non real-time), resolution and objectivity of display (quantification of relative tissue perfusion), specificity of display (false positive incidence), episode of intraoperative decision change based on display, usability of the device by surgeon and support personnel satisfaction, and per use and overall cost in intestinal anastomoses and in comparison to those measured for the use of standard laparoscope or ICG-based visualization systems.

The clinical metrics for secondary objectives on displaying biliary tree include preparation time in workflow, latency of display (real-time versus non real-time), resolution and objectivity of display (quantification of relative tissue perfusion), specificity of display (false positive incidence), episode of intraoperative decision change based on display, usability of the device by surgeon and support personnel satisfaction, and per use and overall cost in LC and in comparison to those measured for the use of standard WLI laparoscope alone or ICG-based visualization systems before and after dissection for the critical view of safety. The display specifically refers the visualization of 7 structures before and after dissection including common bile duct (CD), right hepatic duct (RHD), common hepatic duct (CHD), common bile duct (CBD), cystic common bile duct junction (CCBDJ), cystic gallbladder junction (CGJ), and accessory ducts (AD).

Eligibility:

- All patients undergoing laparoscopic or robot assisted gastrointestinal anastomoses.
- All patients undergoing laparoscopic or robot assisted cholecystectomy

Design:

- This is a feasibility study designed to evaluate safety and feasibility of **ActivSight™** in gastrointestinal anastomoses and cholecystectomy.
- Safety will be determined through clinical assessments and evaluation of any adverse event.
- Feasibility will be determined through technically successful completion of intended visualization.
- Assessment of preliminary efficacy will be performed through analysis of any intraoperative decisions made based on visual display as compared to standard endoscopic approach, or non-inferiority to ICG-based visualization and usability.
- Patients outcome and follow up to Postoperative day 28 will be monitored for clinical outcome.
- Target enrollment for the assessment of intestinal anastomoses is total of twenty patients: 14 evaluable patients with stopping rules that are triggered following the first 6 patients and a comparison group 6 patients in ICG-based treatment.
- Target enrollment for the assessment of biliary tree is total of fourteen patients: 14 evaluable patients with pre-planned use of ICG-based IOC.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 PRIMARY OBJECTIVE

- To determine safety and feasibility of ActivSight™ Imaging in human.

1.1.2 SECONDARY OBJECTIVE

- To provide an assessment of efficacy and usability of ActivSight™ in human based on clinical metrics and intraoperative imaging on tissue perfusion and detection of critical structures such as vascularity or biliary tree.

1.2 BACKGROUND AND RATIONALE

1.2.1 TISSUE PERFUSION AND VASCULATURE AND CURRENT INTRAOPERATIVE ASSESSMENT OPTIONS

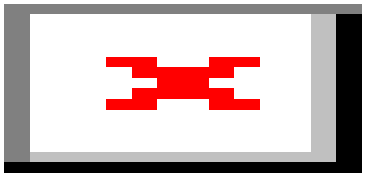
All surgery involves four key steps: incision to access the target tissue, dissection and removal of pathology, reconstruction of anatomy and function, and closure. The integrity of vasculature and tissue perfusion at the operative site remains a critical determinant in expectant tissue healing, avoiding complications and ensuring good clinical outcome [1-9]. This is particularly true in such commonly performed surgical task as a soft tissue anastomosis where two edges of luminal structures including intestine and blood vessel are sutured together to provide a sealed luminal continuity. Anastomosis usually comes during the third reconstructive stage. The critical factors affecting the anastomotic outcome include: the health of local tissue including perfusion status and contamination; physical parameters in anastomotic techniques such as suture bite size, spacing, and tension; anastomotic materials including suture and staples; and human factors such as the surgeon's technical decisions and experience. Recent key advances in surgery such as progressively more minimally invasive surgery (MIS) and robot-assisted surgery (RAS) approaches however, have not addressed these critical determinants, and the overall anastomotic complication rates remain unchanged [1-9].

Most of current vision technology in surgery passively displays various sizes, shapes and contours of surgical anatomy, limited to narrow human color vision range, interpreted by each surgeon's own experience, recall and judgement. Enhanced digital resolution and magnification in endoscopic procedures do not add any functional information, such as perfusion or 'hidden' critical structures. This is the crucial reason that surgical outcomes and complication rates have not further improved, despite the technologic shift [10-18]. These limits cause: (1) missed leaks and necrosis of skin flaps due to the lack of tissue perfusion information; (2) unrecognized injuries to critical blood vessels, bile ducts or ureters due to lack of awareness; and (3) higher rates of perforation and mortality due to lack of tissue depth and vascularity information [10-18]. Up to 6 - 48% of bowel surgery is complicated by leaks, and unintended bile duct and ureter injuries alone cost $2 billion each year [1, 10-18]. The digital endoscopic approach further constrains endoscopic vision pivoted around insertion points while providing the smaller visual field with limited peripheral vision necessitating time consuming repetitious

movements to execute tasks such as suturing. A more precise, quantitative, physiologic display with a clearer target tissue of interest-to-background contrast, tissue health and subsurface tissue information such vascularity and tissue perfusion status would significantly improve the surgeon's operative decisions and the functional outcome for such surgical task.

Today, the most commonly used method of assessing tissue perfusion and critical structures such as vascularity intraoperatively continues to be simple visual inspection of tissue using naked human eyes guided by each surgeons' recall and experience regardless of surgical approach, MIS or open. This 'technique' has not changed over the last two thousand five hundred years [1-5]. The use of X-ray dye and radiologic imaging can provide real-time visualization of vasculature and biliary tree [14,16]. This approach however, has limited utility in everyday use for obvious reasons including radiation exposure, non-real time visualization, capital cost, etc. The use of non-visible light imaging beyond human red-green-blue (RGB) visual range such as near-infrared fluorescence (NIRF) technology has been more recently applied to improve target tissue visualization such as vasculature and biliary tree during surgery [10-18].

The current vision effort to provide additional physiologic or positional information is based on a fluorescent dye, indocyanine green (ICG) which binds non-specifically to plasma phospholipids for detection by infrared camera systems to visualize the tissues, vessels and bile ducts [10-18]. NIR imaging with Indocyanine green (ICG) has been shown to be very effective in displaying blood vessels and graft patency invisible to human eyes intraoperatively, and combining multi-spectral images from several wavelengths can distinguish tissue types and subcutaneous structures arteries and veins and hemoglobin oxygen saturation [10-18]. Hyperspectral imaging beyond the naked eye has the potential to guide optimal surgical tasks including displaying ideal positions and precise placement such as suturing in anastomosis and display these real-time physiologic parameters that are critical to tissue healing and outcome. However, there are currently only a few fluorescent dyes (in fact, only one cleared by FDA to date, namely ICG) approved for clinical use, and requires additional expensive imaging equipment to display ICG [10-18]. The current intraoperative use of fluorescent dye, namely ICG is further complicated by the need for preparation times, costs, dose constraints, short half-cycles, allergic reactions, injection timings, and the inability to conduct multiple-use case [10-18]. Dye-free, intraoperative visualization of critical hidden, sub-surface structures in real-time such as vasculature and tissue perfusion obviate the above disadvantages of dye-based visualization and would be ideal most surgical procedures.



(A) the standard view from a surgical laparoscopic camera, (B) an ICG fluorescence-based perfusion view of the soft tissue, and (C) an ICG-based perfusion overlay.

Laser-speckle-contrast imaging (LSCI) represents a label-free imaging method using coherent monochromatic light where blood flow and tissue perfusion can be detected based on calculated average velocities and red blood cell concentrations [19-24]. LSCI does not require any dye, is non-contact to the tissue of interest, and can distinguish arteries from veins. Activ Surgical Inc. has now combined an innovative form factor and proprietary software into **ActivSight™,** to deliver precise, objective, cost efficient real-time visualization

of blood flow and tissue perfusion intraoperatively for laparoscope-based surgery. A small adaptor that fits between any existing laparoscope and camera systems and a separate light source placed along any current commercial system will deliver objective real-time tissue perfusion and blood flow information intraoperatively. **ActivSight™** has recently demonstrated the superiority to clinically available ICG-based visualization of vasculature and tissue perfusion in intestinal anastomoses in a preclinical model [25].

Interestingly, our preliminary preclinical data also indicate that **ActivSight™** can also readily display an ICG-based visualization of the biliary tree in real time at equivalent or superior to current commercial products. The innovative form factor of **ActivSight™** enables any laparoscopic system for ICG-based visualization at a fraction of the cost of current competitor with minimal disruption to workflow.

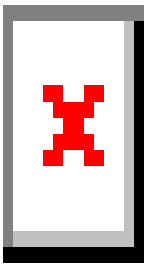
Bile duct injury (BDI) during cholecystectomy remains a serious surgical complication with a significant risk of early death, serious ongoing morbidities including multiple reinterventions requiring prolonged and repeated hospital stay, over a billion dollars in healthcare costs alone in US alone each year [29-31]. The introduction laparoscopic approach despite several benefits, however, resulted in increased incidence of BDI up to 4-folds in some reports. Most major biliary injuries result from misidentification, either misidentification of the common bile duct as duct as the cystic duct or misidentification of an aberrant bile duct. It is increasingly clear that the use of intraoperative cholangiography (IOC) has a significant association with improved survival. The recent state of the art consensus conference on prevention of bile duct injury during cholecystectomy in 2018 strongly recommends that the use of intraoperative biliary imaging to mitigate the risk of BDI during laparoscopic cholecystectomy (LC) in patients with uncertain anatomy or suspicion of BDI and the liberal use of IOC during any LC. Performing IOC laparoscopically however challenges surgeons' skills, is time-consuming, and requires a learning curve to interpret images.

The currently available additional means of visualizing biliary tree intraoperatively are limited to radiation-based fluoroscopy with X-ray contrast dye. These approaches are suboptimal in effectiveness (non-real time, lacks objectivity and specificity), cumbersome to efficient workflow (need for contrast reagent preparation and injection, potential adverse allergic reactions, risk of radiation exposure and/or training requirement), and efficacious utility (need for large capital equipment, space and cost). Recent randomized controlled trial, near-infrared fluorescent cholangiography (NIFC) was statistically superior to white light (WLI) alone visualizing extrahepatic biliary structures during laparoscopic Cholecystectomy. Pre-dissection detection rates were significantly superior in the NIFC group for all 7 biliary structures. After dissection, similar intergroup differences were observed for all structures except CD and CGJ, for which no differences were observed. Increased body mass index was associated with reduced detection of most structures in both groups, especially before dissection. Only 2 patients, both in the WL group, sustained a biliary duct injury [31].

Thus, the intended use of **ActivSight™** is to provide real-time laparoscopic near infrared imaging. **ActivSight™** enables surgeons to visually assess blood vessels, blood flow, related tissue perfusion and critical structures such as biliary tree using near infrared imaging during minimally invasive surgery.

1.2.2 CURRENT STATE OF CLINICAL LSCI USE

Laser speckle contrast imaging (LSCI) is a fast, full-field, inexpensive, and relatively simple imaging method that can give 2-D perfusion maps of large surfaces, and is gaining clinical applications in ophthalmology, rheumatology, neurology, dermatology and oral surgery [18-24]. LSCI is based on the principle that the backscattered light from a tissue that is illuminated with coherent laser light forms a random interference pattern at the detector, the so-called speckle pattern.



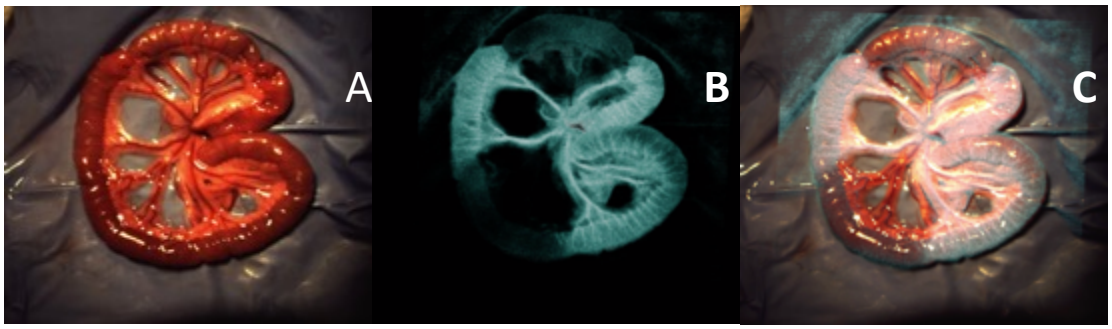
LSCI is extensively used in ophthalmology and Perfusion measurements of the choroidal circulation, critical for the diagnosis of various diseases such as glaucoma, retinopathy, and macular degeneration [18-24]. In rheumatology, the use of LSCI to monitor the dynamic vascular reactivity and blood flow in patients affected by Raynaud's phenomenon including SSc patients clearly illustrate the utility of LSCI in detecting vascularity and tissue perfusion: [LASCA image of right hands and related NVC pictures in (a) a healthy subject, and in SSC patients with (b) "early, (c) "active", or (d) "late" pattern of microangiopathy (NVC picture magnification 200×). © 2019, with permission from BMJ]. Similarly, Lindahl et al. reported a study using LSCI on pediatric burn injuries trying to predict the outcome of the burn at 14 days postburn, correlating images that can predict wound healing [18-24]. LSCI is also used in dermatology for monitoring port wine stain (PWS) birthmarks. PWS are progressive vascular malfunctions with locally increased blood flow.

Although the use of LSCI in three patients undergoing extracranial–intracranial bypass procedures have been reported in neurosurgery where verifying cerebral blood perfusion levels at presurgical levels are correlated to postsurgical tissue viability, however, the real translation from the experimental setting to clinical practice as a standard of care has yet to come [18-24]. Similarly, a limited number of clinical case reports on gastric microvascular perfusion in gastric tube reconstructions documented ischemic areas in reconstructed gastric tube, and confirmed that LSCI can be of help in identifying ischemic areas to potentially reduce anastomotic leakage, which remains a major complication within gastrointestinal surgery [18-24].

1.2.3 PRECLINICAL DATA ON ACTIVSIGHT

The **ActivSight™** System seeks to solve these critical unmet needs of unrecognized/unintended injuries to critical structures such as blood vessels and biliary tree, and tissue perfusion status by providing surgeons with clinically useful, real-time images of blood flow, tissue perfusion and critical structures. **ActivSight™** an intelligent intraoperative vision based on hyperspectral imaging will change the surgical vision paradigm from passive display limited to the narrow human vision spectrum to physiologic and functional tissue information, including hidden subsurface structures. **ActivSight™** will be easy to use and provide real-time display of physiologic perfusion status at tissue level, hidden subsurface vascular anatomy, and tissue classification, such as arterial versus venous structures. This will be enabled without the use of chemical signal enhancers, such as fluorophores. The superiority in function and utility of **ActivSight™** mitigate the limitations of pharmacokinetics of drugs and patient factors such as total allowable dose per use. It also mitigates limited tissue signal efficacy due to the lack of tissue specificity and optical properties, including depth of visualization inherent to any ICG dye -based system [16,25].

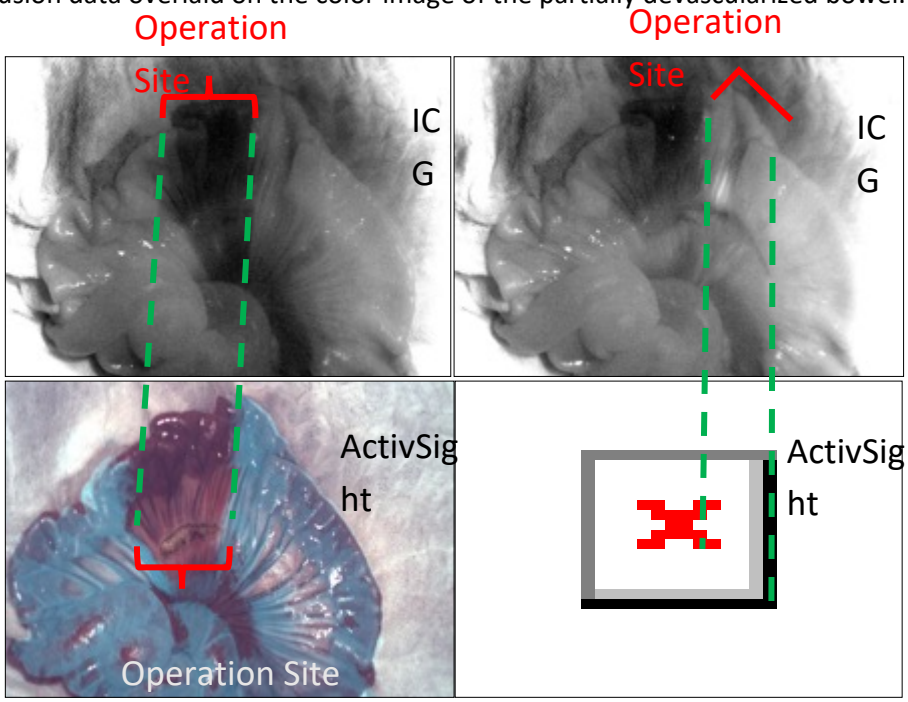
ActivSight™ provides the following benefits:
1. **Quantitative Images: ActivSight™** is based on laser speckle technology (detailed in Data Processing section) and, as such, provides objective information specific to blood flow and tissue perfusion.
2. **No contrast agent: ActivSight™** can be used by the physician at any moment during the procedure to visualize blood flow and tissue perfusion
3. **Real-time: ActivSight™** will show current blood flow and tissue perfusion no matter how many times the blood supply is altered during the procedure, as the system can be turned on and off throughout the procedure.
4. **Lower patient risk**: The risk of adverse events related to contrast agents is eliminated over systems providing similar imaging capabilities, as no contrast agent is required for use of **ActivSight™** .
5. **Usability and cost benefit:** In clinical applications using ICG, **ActivSight™** will display critical structures equivocal or superior to current clinical predicates without interruption to workflow and need for significant capital equipments.



A) **ActivSight™ Standby** – When **ActivSight™** visualization is in standby mode, the **ActivSight™** System will not interfere with the normal operation of the laparoscopic camera system to which it is attached. Above is a color view from a traditional laparoscopic visualization system of an intestine where blood flow has been intentionally cut off in several regions of tissue. In this example, the **ActivSight™** device is attached, but it is not supplementing the image obtained by the surgical laparoscopic system.

B) **ActivSight™ Perfusion View** – This display will show, in real-time, where there is active blood flow in the visualized tissue and vessels. By showing the areas of active blood flow, the Operating Room staff can clearly see small vessels and watershed areas that may be hard to see with the naked eye when only the color surgical video is being displayed. As can be seen in the image below, the portions of bowel that have been cut off from blood supply are darker while areas with active perfusion and blood flow are brighter. This view is achieved by laser speckle imaging, which is discussed in greater detail below.

C) **ActivSight™ Overlay** – In this view, the display will show a view of the real-time surgical video, overlaid with the perfusion data. This view will be primarily used to perform real-time evaluation of perfusion while manipulating soft tissue (with or without blood flow) during a surgical procedure. The image above shows perfusion data overlaid on the color image of the partially devascularized bowel.

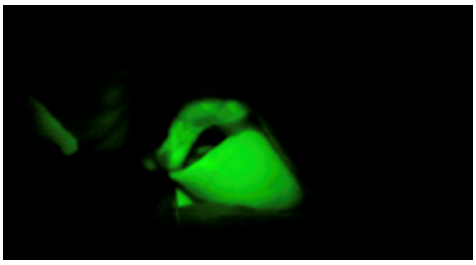


The panel on the right demonstrate: real-time display, the precision, and no false-positive visualization of **ActivSight™** in comparison to commercial ICG-based visualization of intestinal vascularity and tissue perfusion. ICG-based visualization is not real time. The blood flow can be visualized only while the contrast agent is in the vasculature (short time window post injection). Moreover, the contrast agent's fluorescence property cannot be turned off. ICG trapped in a clamped vessel will continue to fluoresce, even though the segment of the bowel has been completely de-vascularized, and the downstream tissue is no longer perfused. Since ICG is not specific to blood flow, ICG diffuses out of the vasculature into the interstitial fluid of the surrounding tissue, emitting a signal that may lead to confusion.

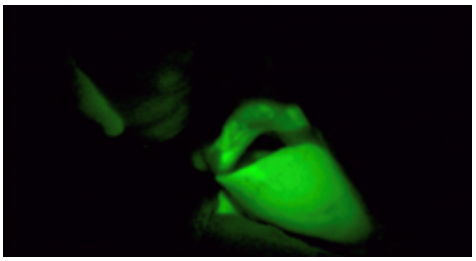
We recently demonstrated the superiority of a novel endoscopic visualization system, **ActivSight™** to ICG based visualization of vasculature and perfusion [25]. We reported the utility as measured by the ease of use and the real-time visualization, and the efficacy measured by relative quantification of perfusion and the occurrence of false positive display. *In vivo* testing consisted of bowel anastomoses followed by serial devascularizations of mesentery in porcine model was used (n = 14). The objectivity of perfusion signal detected by the **ActivSight™** system was validated using commercially available FDA-approved laser doppler system (Perimed PeriFlux System 5000). We observed that **ActivSight™** detected real-time parenchymal perfusion and mesenteric blood flow in the intestine at the anastomoses with an average 150ms processing latency in contrast to commercial ICG fluorescent systems, with >300 folds the latency of **ActivSight™** (excluding ICG preparation time) ($p<0.01$). Since ICG can be used accurately only once upon devascularization of the bowel, all commercial ICG systems consistently displayed (100%) false-positive fluorescent images following serial devascularization due to the retention of ICG in the ischemic segments while **ActivSight™** displayed none. Fluorescence intensity function further accentuated the false positive display in all commercial systems. The use of **ActivSight™** had no impact on clinical workflow associated with no ICG prep and injection time. The tissue perfusion signal intensity detected by **ActivSight™** correlated significantly with the perfusion data (blood pressure and flow) measured using commercially available FDA-approved laser doppler system ($r = 0.87$; $p<0.001$). We

concluded that **ActivSight™** is superior to ICG-based fluorescence imaging in efficiency (real-time display, minimal latency, no preparation time), effectiveness (objectivity, no false positive display) and usability (no drug injection and reduced cost) in displaying tissue perfusion and vascularity [25].

Moreover, our recent preclinical study demonstrates that **ActivSight™** can visualize the critical biliary structures when ICG is used during laparoscopic cholecystectomy. The use of ICG in IOC is already approved for clinical use, and the recent randomized controlled trial, the use of ICG in near-infrared fluorescent cholangiography (NIFC) was superior to white light (WLI) alone visualizing extrahepatic biliary structures during laparoscopic Cholecystectomy [10]. The recent state of the art consensus conference on prevention of bile duct injury during cholecystectomy in 2018 strongly recommends that the use of intraoperative biliary imaging to mitigate the risk of BDI during laparoscopic cholecystectomy (L C) in patients with uncertain anatomy or suspicion of BDI and the liberal use of IOC during any LC.



Existing ICG Camera System



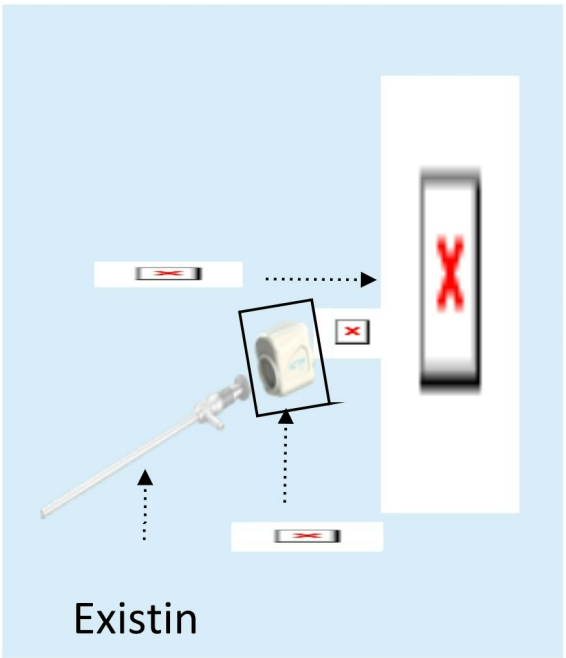
ActivSight's view of ICG

1.2.4 ACTIVSURGICAL INC. HYPERSPECKLE VISUALIZATION DEVICE FOR VASCULARITY AND PERFUSION

1.2.4.1 DEVICE DESCRIPTION AND INDICATIONS FOR USE

ActivSight™ System

The **ActivSight™** System is intended as an accessory to existing surgical laparoscopic camera systems. As shown in the adjacent figure, the **ActivSight™** System is comprised of two components: (1) the reusable **ActivSight™** Adapter with Sensing Electronics; and (2) the reusable **ActivSight™** Control Unit (each component is discussed in greater detail below).



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The **ActivSight™** Adapter attaches in-line between the third-party surgical camera and the third-party laparoscopic coupler, as shown in the diagram. The purpose of the **ActivSight™** Adapter is to retain surgical laparoscopic functionality while diverting key wavelengths of light to the connected **ActivSight™** Sensing Electronics for processing. The

ActivSight™ Adapter is compatible with the standard laparoscopic coupler and the standard laparoscopic camera c-mount. The **ActivSight™** Control Unit: 1) controls and emits coherent near-infrared light from the **ActivSight™** System's light source (within the Control Unit); and 2) processes video streams from the **ActivSight™** Sensing Electronics and the third-party surgical camera system to produce the system's

ActivSight™ Adapter
The **ActivSight™** Adapter (see above) is a component that attaches between a standard autoclavable surgical laparoscope (via coupler) and the third-party surgical camera. The **ActivSight™** Adapter mates to the third-party coupler and to the third-party camera via standard C-mount connection. The **ActivSight™** Adapter provides a mechanical interface for the **ActivSight™** Sensing Electronics at a 90-degree offset from the imaging path. The **ActivSight™** Adapter is reusable and is covered by a standard laparoscopic camera sterile drape during a surgical procedure. Besides the mounting interfaces, the

ActivSight™ Adapter is comprised of optical elements within a plastic or stainless-steel enclosure. Functionally, the **ActivSight™** Adapter is responsible for diverting longer wavelengths of light (NIR waveband) in the imaging path of the laparoscope to the **ActivSight™** Sensing Electronics while passing shorter wavelengths of light (Visible waveband) to the standard third-party surgical camera. The shorter wavelengths of light are in the visible spectrum and are passed to the third-party surgical camera to preserve its ability to produce an accurate color image of the patient's anatomy. There shall be no change to the performance of the third-party laparoscopic camera system. The longer wavelengths of light are in the near-infrared (NIR) spectrum and are diverted to the **ActivSight™** sensing electronics in order to observe blood flow and tissue perfusion with additional software processing computed within the **ActivSight™** Control Unit.

ActivSight™ Sensing Electronics
The **ActivSight™** Sensing Electronics module is contained within the **ActivSight™** Adapter. The **ActivSight™** Sensing Electronics module is comprised of 1) electronics including an imaging sensor, 2) a plastic enclosure, 3) a cable to connect the sensing electronics to the **ActivSight™** Control Unit.

The electronics within the **ActivSight™** Sensing Electronics module have the following features:

1. 60 frames-per-second (FPS) video stream
2. Monochrome sensing at a 1920x1080 (1080P HD) pixel resolution
3. CMOS imaging sensor with higher quantum efficiency at NIR wavelengths (between 750nm-1000nm) compared to typical CMOS camera sensors. This results in more efficient imaging at NIR wavelengths without requiring an excessively high-power light source.
4. A one-time-programmable (OTP) memory device for software expiration. The software will write to the memory chip when the device is expired and will prevent re-use on another patient.
5. A button, easily accessible by the user, to toggle between the imaging modes (**ActivSight™** Standby, **ActivSight™** Perfusion View, **ActivSight™** Perfusion Overlay).

ActivSight™ Control Unit
The **ActivSight™** Control Unit (ACU) has the following high-level functions: illumination, data capture, data processing, and image display.

Illumination
The ACU generates narrow bandwidth coherent NIR light that has a discretely programmable wavelength between 750nm and 1000nm. The ACU provides an input on the front of the box to attach a third-party surgical laparoscope light source that feeds the ACU with broadband white light. The white light and NIR light are mixed within the ACU and then relayed to the surgical site by means of a single light guide cable that is attached to the laparoscope. Although both the white light and NIR light are mixed together and simultaneously illuminate the surgical field, the **ActivSight™** Adapter ultimately filters the reflected image so that the third-party camera observes only the reflected response of the white light and the **ActivSight™** Sensing Electronics observe only the reflected response of the coherent NIR light.

Data Capture
The ACU captures two streams of video data: 1) a video stream from the CMOS sensor within the **ActivSight™** Sensing Electronics module; and 2) a video input that originates from the third-party surgical camera control unit. The third-party video stream is captured by connecting the video output of the third-party camera control unit to the ACU.

Data Processing
The ACU contains a computer processor that receives the video stream from the CMOS sensor within the **ActivSight™** Sensing Electronics and applies software processing on the incoming data. To visualize blood flow and tissue perfusion, the **ActivSight™** System uses algorithms related to laser speckle contrast imaging (LSCI) that can provide information about particle velocity in an area. The NIR coherent light source described above illuminates the surgical scene in such a way that the surface scatters the light and an interference pattern – speckle pattern – is generated on the sensor. The speckle pattern remains constant for stationary objects while moving particles in the scene result in varying interference thereby allowing the software to identify flowing blood. Software image processing is used to highlight areas of the scene with greater velocity (such as blood flowing through a vessel).

Image Display
The ACU produces a perfusion view that depicts the regions of tissue that have higher blood flow and tissue perfusion. As described in the section "Pre-Clinical Data of **ActivSight™**," the resulting perfusion map can be hidden from the user ((1) **ActivSight™** Standby mode) or shown to the user ((2) **ActivSight™** Perfusion View mode or (3) **ActivSight™** Overlay mode). When the overlay is enabled, the standard third-party laparoscopic color image is shown with an overlay of the perfusion data. Video generated by the ACU in either of the three modes can be visualized on a medical-grade surgical monitor connected to the Video out port of the ACU.

Reprocessing
The Light Engine is not intended to come into contact with the patient. It may be cleaned, but not sterilized. The imaging module is used in the sterile field and therefore shall be cleaned and sterilized prior to every use.

Cleaning the Light Engine
Should the Light Engine need cleaning, follow the warnings, cautions, and instructions below. The user shall provide the mild detergent (or standard disinfectant) and sterile cloth required for cleaning.

Cleaning, Disinfecting, and Sterilizing the Imaging Module
These reprocessing instructions are provided in accordance with ISO 17664, AAMI TIR12, AAMI TIR30, AAMI ST79, and AAMI ST81. While they have been validated by Activ Surgical Inc. as being capable of preparing the device for re-use, it remains the responsibility of the processor to ensure that the reprocessing, as actually performed (using equipment, materials, and personnel in the reprocessing facility), achieves the desired result. This normally requires routine monitoring and validation of the facility's reprocessing procedures. Activ Surgical recommends users observe these standards when reprocessing medical devices.

The Activ Surgical Inc. **ActivSight™** pre-submission documentation to the US FDA clearly indicate that pre-clinical data would suffice the clearance for the US market through FDA's 510(k) premarket notification review process given the substantial equivalence to the submitted predicates identified by Activ Surgical Inc.

Within this proposed clinical study, the **ActivSight™** is indicated and tested for visualization of vascularity and tissue perfusion during intestinal anastomoses.

1.2.5 RISK MITIGATION STRATEGY

Given the innovative design of **ActivSight™** as an interposing adaptor between any commercial endoscope and camera systems with no direct patient contact, there is no biocompatibility issue. Our preclinical testing indicates that there is also a minimal or imperceptible loss of RGB signals (99.8% transmittance of WLI), image quality and usability of the third-party endoscopic device. However, in the case of any significant loss of **ActivSight™** image quality in display, the default mode is to revert to standard RGB video mode and the manual assessment of tissue vascularity and perfusion relying on the surgeon. An alternative is to switch to an ICG-based NIR-mediated visualization system with minimal disruption to the workflow.

The safety of monochromatic coherent light source is well documented. The wavelength range of **ActivSight™** falls well within the approved safety of commercial ICG/NIR endoscope-camera systems including ophthalmologic concerns and the FDA clearance for patient safety for any thermal injury.

The sterilization specification of **ActivSight™** meets the reprocessing requirements in accordance with ISO 17664, AAMI TIR12, AAMI TIR30, AAMI ST79, and AAMI ST81. However, if there are any concerns for breach of sterility, a standard translucent sterile plastic sleeve will drape over the endoscope-adaptor-camera-cable connection.

1.2.6 PROPOSED CLINICAL TRIAL RATIONALE

ActivSight™ offers an innovative form factor and proprietary software to deliver precise, objective, cost efficient real-time visualization of blood flow and tissue perfusion intraoperatively for laparoscope-based surgery. A small adaptor that fits between any existing laparoscope and camera systems and a separate light source placed along any current commercial system will deliver objective real-time tissue perfusion and blood flow information intraoperatively. In comparison to the current practice of assessing tissue vascularity and perfusion based on either visual inspection by naked eye or

cumbersome ICG-based system, our **hypothesis** is that **ActivSight™** is a safe and feasible vision technology, and is non-inferior to displaying tissue perfusion and critical structure to ICG-based Near Infrared (NIR) mediated predicate devices in clinical practice during intestinal anastomoses and laparoscopic cholecystectomies. With potential for superiority in efficacy and utility, **ActivSight™** offers an extremely attractive option that may replace the current standard therapy options for displaying tissue vascularity and perfusion intraoperatively in all anastomoses.

The **Primary Objective** is to determine safety and feasibility of **ActivSight™** in displaying tissue perfusion in gastrointestinal anastomoses including colorectal and bariatric surgery. The **Secondary Objective** is to determine the efficacy of **ActivSight™** in; (1) displaying tissue vascularity and perfusion during gastrointestinal anastomoses and in comparison to ICG; and (2) displaying biliary tree during laparoscopic cholecystectomy using ICG-based IOC.

The clinical metrics for secondary objectives on tissue perfusion and vascularity include preparation time (single versus multiple injections of ICG), latency of display (real-time versus non real-time), resolution and objectivity of display (quantification of relative tissue perfusion), specificity of display (false positive incidence), episode of intraoperative decision change based on display, usability of the device by surgeon and support personnel satisfaction, and per use and overall cost in intestinal anastomoses and in comparison to those measured for the use of standard laparoscope or ICG-based visualization systems.

The clinical metrics for secondary objectives on displaying biliary tree include preparation time in workflow, latency of display (real-time versus non real-time), resolution and objectivity of display (quantification of relative tissue perfusion), specificity of display (false positive incidence), episode of intraoperative decision change based on display, usability of the device by surgeon and support personnel satisfaction, and per use and overall cost in LC and in comparison to those measured for the use of standard WLI laparoscope alone or ICG-based visualization systems before and after dissection for the critical view of safety. The display specifically refers the visualization of 7 structures before and after dissection including common bile duct (CD), right hepatic duct (RHD), common hepatic duct (CHD), common bile duct (CBD), cystic common bile duct junction (CCBDJ), cystic gallbladder junction (CGJ), and accessory ducts (AD).

We will enroll the total of 20 patients; 14 in **ActivSight™** group will initially enroll 6 patients followed by additional 8 patients. Six patients matched for demographics and indications will be enrolled in ICG-based NIR-mediated visualization of tissue perfusion and vascularity as a comparison. Stopping rules will be triggered if it is predicted that stringent adverse event rate will be 20% or higher by Day 28. Individual trigger thresholds are based on the posterior probability that adverse event rate associated with the use of technology rate exceeds pre-specified rate or adverse event rate (20%) is greater than 0.90. Namely, let q = Prob(adverse event| data) in the trial which follows a beta distribution with a non-informative beta (.30, .70) prior, we will stop the trial for review if $Pr(q > \Gamma \mid data) > .90$ where Γ is the adverse rate (20%). Specifically, the trial will be terminated early if [# patients with adverse event] / [number of patients] is 2/4, 3/7, 4/11 or 5/15. Note that, for example, the requirement to stop at 2/4 implies that the trial will be stopped at 2/3. The study would also be suspended if there were at least 2 patients in whom any device technical issues ensue and are not completed. However, if the technical obstacles are overcome, the study may resume, pending input of the Medical Safety Monitor Committee.

The initial cohort will provide limited information on adverse events, as even one adverse event would imply a very high upper bound of an exact confidence level about the rate of adverse event. However,

with the second cohort if 3 of 14 evaluable patients (~21%) experience an application limiting adverse event, the upper bound of an 85% confidence interval is 0.50; implying a confidence of 85% that there is no worse than a 50% probability that an application limiting adverse event would occur in a given patient. Similar considerations will be applied to the technical feasibility of the application overall. Thus, a sample size of 14 patients is expected with a planned 20% in-evaluable rate.

Similarly, for the secondary objective of determining **ActivSight™** displaying biliary tree during laparoscopic cholecystectomy using ICG-based IOC, we will apply the same safety and feasibility rationale as described above.

Adverse events will be summarized descriptively and tabulations on the type, severity, and relationship to device use will be performed.

2 ELIGIBILITY, ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 INCLUSION CRITERIA

- All patients age ≥ 18 years old undergoing laparoscopic or robot assisted intestinal anastomoses, or all patients age ≥ 18 years old who are planned for laparoscopic cholecystectomy; spoken command and literacy in the native language spoken at each participating center; ability to understand and follow study procedures; and having provided signed consent.
- Diagnosis:
    - All patients with a clinical suspicion and diagnosis of benign or malignant, small or large bowel lesions requiring surgical resection.
    - All patients with clinical suspicion and diagnosis of symptomatic cholithiasis or cholecystitis planned for cholecystectomy.
    - Typical imaging as per standard workup findings including US, CT and/or MRI. Plain radiographs and contrast imaging may be obtained by referring physicians and are helpful for confirming the clinical diagnosis.
    - Any bariatric patients undergoing gastric sleeve or bypass.
    - Any pediatric patient undergoing laparotomy for NEC

- Location of pathology or resected segment:

- Target lesions can be located in any fore-, mid- or hindgut segments requiring reconstruction and anastomoses.

- Prior therapy:
    - Patients with prior surgery are eligible for enrollment.

- Laboratory:
    - Hemoglobin > 9 g/dL
    - Platelet count ≥75,000/µL (may receive transfusions)
    - Normal PT, PTT and INR < 1.5 x ULN (including patients on prophylactic anticoagulation)
    - Renal function: Age-adjusted normal serum creatinine derived from Schwartz formula for estimating GFR by the CDC OR a creatinine clearance ≥60 mL/min/1.73 m2 for safe
    - Adequate pulmonary function: Defined as no dyspnea at rest, and a pulse oximetry >94% on room air if there is clinical indication for determination.

2.1.2 EXCLUSION CRITERIA

- There is no exclusion criteria for **ActivSight™** for gastrointestinal resection.
- Patients assigned to FDA cleared ICG-based visualization are contraindicated for any chronic renal dysfunction, potential drug interaction, history of allergy to ICG or anaphylaxis, and pregnancy.
- Patients eligible for cholecystectomy, exclusion criteria include known allergy to iodides; known history of cholangitis, pancreatitis, prior common bile duct injury, coagulopathy or known, pre-existing liver disease; pregnancy or breast-feeding; or being of reproductive age with pregnancy possible and not ruled out.
- Patients currently in any investigational agents.

2.1.3 REGULATORY

- Informed consent: All patients or their legal guardians (if the patients is <18 years old) must sign a document of informed consent indicating their understanding of the investigational nature and the risks of this study before any protocol related studies are performed.
- DURABLE POWER OF ATTORNEY (DPA): Patients ≥18 years of age will be offered the opportunity to assign a DPA so that another person can make decisions about their medical care if they become incapacitated or cognitively impaired.

2.2 PRE-TREATMENT EVALUATION

2.2.1 GENERAL EVALUATION

- History and Physical Examination: documentation of measurable disease and signs and symptoms.
- Height, weight and body surface area: must be recorded.
- Vital signs: including blood pressure measured with age appropriate cuff, must be recorded and monitored intraoperatively

2.2.2 LABORATORY EVALUATION

Pre-treatment laboratory tests as a part of routine surgical work up should be performed within 30 days prior to treatment.

Safety and Feasibility of ActivSight™ Laser speckle imaging in visualization of tissue perfusion and critical structures in human

- Hematology: complete blood count. PT, PTT and INR.
- Chemistries: BUN, creatinine, electrolytes, calcium, magnesium, phosphorus.

2.3 PATIENT REGISTRATION

Prior to patient registration, patient eligibility must be confirmed with Drs. Erik Wilson and Dr. Peter Kim; Activ Surgical Inc. 840 Summer Street Suite 108 Boston MS 02127. Phone: 617-333-8162or email: pkim@activsurgical.com. When contacting Drs. , information about all entry criteria (e.g., laboratory results) must be available to allow for verification of eligibility (see Section 2.1).

3 IMPLEMENTATION OF STUDY DESIGN

3.1 STUDY DESIGN

3.1.1 OVERALL ASSESSMENT OF, FEASIBILITY, SAFETY AND PRELIMINARY EFFICACY

This is an early feasibility trial to determine safety and feasibility of **ActivSight™** during gastrointestinal anastomoses. The **Secondary Objective** is to determine the efficacy of **ActivSight™** in; (1) displaying tissue vascularity and perfusion during intestinal anastomoses; and (2) displaying biliary tree during laparoscopic cholecystectomy using ICG-based IOC. Measurable clinical metrics for the secondary objective include preparation time (single versus multiple injection of ICG), latency of display (real-time versus non real-time), resolution and objectivity of display (quantification of relative tissue perfusion), specificity of display, episode of intraoperative decision change, usability by surgeon and support personnel satisfaction, and per use and overall cost.

The total of 20 patients will be enrolled; 14 in **ActivSight™** group will initially enroll 6 patients, followed by additional 8 patients. Six patients clinically matched for demographics and indications will be enrolled in ICG-based NIR-mediated visualization of tissue perfusion and vascularity as a comparison. Stopping rules will be triggered if it is predicted that stringent adverse event rate will be 20% or higher by Day 28.

For the feasibility of **ActivSight™** in displaying biliary tree as IOC, fourteen patients planned for laparoscopic cholecystectomy and ICG-based IOC using study site commercial ICG visualization system will be enrolled. The comparison to those measured for the use of standard ICG-based visualization systems versus **ActivSight™** , before and after dissection for the critical view of safety and specific visualization of 7 structures including common bile duct (CD), right hepatic duct (RHD), common hepatic duct (CHD), common bile duct (CBD), cystic common bile duct junction (CCBDJ), cystic gallbladder junction (CGJ), and accessory ducts (AD) will be performed.

The indication and duration of use of **ActivSight™** will be at each surgeon's discretion during eligible procedures, similar to the indication of ICG-based visualization system for tissue perfusion and vascularity check. For the perfusion feasibility study, **ActivSight™** will be: [1] on 2 minutes before

resection of the pathology; [2] at and during anastomoses; and [3] following completion of anastomoses for verification of tissue vascularity and perfusion. For the comparison of **ActivSight™** in displaying biliary tree as IOC, similar amounts of time as per operating PI surgeon will be allocated to visualize the critical view of safety and 7 proposed structures. **ActivSight™** versus commercial ICG-based visualization system will be alternated in eligible fourteen patients with minimize flow dependent ICG intensity.

Technical feasibility will be assessed based on satisfactory intraoperative real-time display of tissue perfusion and critical structures of intended area of interests, concordance/discordance with RGB-based or ICG-based assessments. **Clinical efficacy** will be established by determining measurable clinical metrics including ICG preparation time (single versus multiple injection of ICG), latency of display (real-time versus non real-time) between ICG and **ActivSight™**, resolution and objectivity of display (quantification of relative tissue perfusion), specificity of display (false positive rate following multiple injection or intensity gating), episode of intraoperative decision change if any between **ActivSight™** and ICG, usability by surgeon and support personnel satisfaction on 5 point Likert scale, and per use and overall cost of each technology.

Safety will be defined through clinical assessments and evaluation of use related adverse events intraoperatively and a routine follow up at 28 days following surgery using the criteria listed below. **ActivSight™** will be deemed safe if hardware (adaptor or light source)-related major (serious) adverse event is encountered in the treated patients and if less than 2 hardware-related minor adverse events are encountered as defined below.

Patient eligibility and imaging will be reviewed and determined by the PI and the study team that consists of members from PIs, a team of engineering faculty with expertise in speckle technology. Patients will undergo standard procedure for laparoscopic or robot assisted surgical resection without any change to local customary protocol. All patients under general anesthesia will be under continuous supervision for observation and cardiac monitoring to assess vital sign changes. There will be no changes to existing pre- and post-operative procedure and protocol except intraoperative visualization for tissue vascularity and perfusion by either **ActivSight™** or ICG-based system. Patients will be discharged in usual manner and time frame as per discretion of supervising surgeon if they do not have any major adverse events and meet routine discharge criteria. All patients will receive a follow up telephone call the next day and will be seen in follow up clinic at postoperative 28 day. Target enrollment is 20 + 14 evaluable patients at the study site, and we anticipate that enrollment will take up to 2 weeks.

Study Design and Statistical Consideration

This trial will initially enroll 6 patients followed by additional 8 patients. Stopping rules will be triggered if it is predicted that the toxicity rate will be 20% or higher by Day 28. Individual trigger thresholds are based on the posterior probability that Treatment Limiting Toxicity (TLT) rate exceeds pre-specified rate or target toxicity rate (20%) is greater than 0.90. Namely, let q = Prob(toxicity| data) in the trial which follows a beta distribution with a non-informative beta (.30, .70) prior, we will stop the trial for review if $Pr(q > \Gamma \mid data) > .90$ where Γ is the adverse event rate (20%). Specifically, the trial will be terminated early if [# patients with adverse event rate] / [number of patients] is 2/4, 3/7, 4/11 or 5/15. Note that, for example, the requirement to stop at 2/4 implies that the trial will be stopped at 2/3. The study would also be suspended if there were at least 2 patients in whom to the device technical issues the treatment is not completed. However, if the technical obstacles are overcome, the study may resume, pending input of the Medical Safety Monitor Committee.

The initial cohort will provide limited information on adverse events, as even one adverse event would imply a very high upper bound of an exact confidence level about the rate of adverse event. However, with the second cohort if 3 of 14 evaluable patients (~21%) experience an application limiting adverse event, the upper bound of an 85% confidence interval is 0.50; implying a confidence of 85% that there is no worse than a 50% probability that an application limiting adverse event would occur in a given patient. Similar considerations will be applied to the technical feasibility of the application overall. Thus, a sample size of 14 patients is expected with a planned 20% in-evaluable rate.

Adverse events will be summarized descriptively and tabulations on the type, severity, and relationship to application will be performed and any changes of outcomes from baseline on follow up will be examined using the nonparametric Wilcoxon rank test.

Outcome: The primary endpoint of safety and feasibility will be determined intraoperatively. The criteria for an adverse event include the following complications that are at least possibly related to **ActivSight™** application: i) major technical failure to display perfusion and critical structures reverting to default mode of RGB, ii) any mechanical or electric issue with the hardware, iii) significant reduction of third party hardware light intensity, iv) any minor discomfort to the eye, v) any difference in clinical outcome at postoperative day 28 follow up, vi) any tissue thermal change, vii) additional decision change following completion of anastomosis following **ActivSight™** application.

3.1.2 DEFINITION OF ADVERSE EVENT

Adverse events will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 (http://ctep.cancer.gov), and the Clavien-Dindo classification of surgical classification [26,27]. Possible toxicities that are usually considered usability or application limiting in any phase 1 drug/device trials include: i) Major bleeding requiring surgery; ii) Permanent motor nerve deficit; iii) Spinal cord Injury; iv) Skin burn requiring a graft; v) Osteomyelitis; vi) Septic arthritis; vii) Renal insufficiency requiring dialysis. It is unlikely that any of these toxicities will occur with the use of **ActivSight™**.

Major adverse events limiting the use or requiring intervention are listed below.

- Major technical failure to display vascularity and perfusion reverting to default mode of RGB
- Any mechanical or electric issue with the hardware
- Significant reduction of third-party hardware light intensity
- Any minor discomfort to the eye
- Any difference in clinical outcome at postoperative day 28 follow up
- Any tissue thermal change due to thermal effect of the NIR
- Additional decision change following the completion of anastomosis using **ActivSight™**

3.1.3 DURATION OF APPLICATION

Duration of **ActivSight™** use consists of standard RGB visualization of intraoperative scenes and on demand use with the **ActivSight™** overlay views as per operating surgeon's discretion.

3.2 ON STUDY EVALUATIONS

3.2.1 GENERAL EVALUATION

Clinical and technical utility including usability and any impact on standard workflow will be evaluated intraoperatively based on satisfactory intraoperative real-time display of tissue vasculature and perfusion of intended area of interests, concordance/discordance with RGB-based or ICG-based assessments, measurable clinical metrics including ICG preparation time (single versus multiple injection of ICG), latency of display (real-time versus non real-time) between ICG and **ActivSight™**, resolution and objectivity of display (quantification of relative tissue perfusion), specificity of display (false positive rate following multiple injection or intensity gating), episode of intraoperative decision change if any between **ActivSight™** and ICG, usability by surgeon and support personnel satisfaction on 5 point Likert scale, and per use and overall cost of each technology, and Day 28 follow up assessment of clinical status.

3.3 DISCONTINUATION OF PROTOCOL

3.3.1 LACK OF EFFICACY

Individual trigger thresholds are based on the posterior probability that adverse event rate associated with the use of technology rate exceeds pre-specified rate or adverse event rate (20%) is greater than 0.90. Namely, let q = Prob(adverse event| data) in the trial which follows a beta distribution with a non-informative beta (.30, .70) prior, we will stop the trial for review if $Pr(q > \Gamma \mid data) > .90$ where Γ is the adverse rate (20%). Specifically, the trial will be terminated early if [# patients with adverse event] / [number of patients] is 2/4, 3/7, 4/11 or 5/15. Note that, for example, the requirement to stop at 2/4 implies that the trial will be stopped at 2/3. The study would also be suspended if there were at least 2 patients in whom any device technical issues ensue and are not completed.

The initial cohort will provide limited information on adverse events, as even one adverse event would imply a very high upper bound of an exact confidence level about the rate of adverse event. However, with the second cohort if 3 of 14 evaluable patients (~21%) experience an application limiting adverse event, the upper bound of an 85% confidence interval is 0.50; implying a confidence of 85% that there is no worse than a 50% probability that an application limiting adverse event would occur in a given patient.

3.3.2 OTHER REASONS

- Patient withdrawal of consent. Reasons must be noted on the patient's record.
- Lost to follow up

4 SUPPORTIVE CARE ISSUES

Appropriate antibiotics, blood product support, and general supportive care measures will be used as clinically indicated.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

5.1.1 SOURCE DOCUMENTS

Source documents are defined as original documents, intraoperative video, data and records. This may include hospital records, clinical and office charts, laboratory data/information, patients' diaries or evaluation checklists, pharmacy dispensing and other records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, X-rays, US, CT and MRI scans.

The investigator will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s), providing direct access to source data documents.

5.1.2 CASE REPORT FORMS

OpenClinica (OpenClinica, LLC, Waltham, MA, USA) is a web-based data entry system utilizing a high security environment. The underlying storage facility will be PostgreSQL, whose structure permits the linking of subject information across all tables (https://openclinica.com/), similar to other relational databases. OpenClinica uses secure socket layers to ensure that the data are well-protected. OpenClinica is 21 CFR Part 11 compliant, and hence includes audit trails for all data entry and corrections and is appropriate for storing and tracking data for IDE purposes.

Data may be either directly entered through the website or will be captured on paper case report forms created by the OpenClinica application and the site will then enter this data into OpenClinica after the visit. The principal investigator or research coordinator will review the eCRFs for completeness and accuracy. In addition, to ensure accuracy, the data will go through some validity checks at the time of entry (mostly range checks and date validity checks). Routine edit checks will run more complex queries. The intent in this approach is to allow sites to complete data entry promptly, and not slow them down except for very gross errors, and to query shortly thereafter should the data at the site show inconsistency. The data management staff will monitor data to ensure timely and uniform collection.

5.1.3 DATA QUALITY ASSURANCE

The CRA/Coordinator will monitor each patient's data set throughout the study. Source document review will be made against entries on the eCRF and a quality assurance check will be performed to ensure that the investigator is complying with the protocol and regulations. In addition, after eCRFs are completed by the CRA, review of the data will be conducted by an independent physician.

5.2 RESPONSE CRITERIA

- Technical feasibility metrics include satisfactory intraoperative real-time display of tissue vasculature and perfusion of intended area of interests, concordance/discordance with RGB-based or ICG-based assessments, latency of display (real-time versus non real-time) alone and comparison to ICG and **ActivSight™**, resolution and objectivity of display (quantification of relative tissue perfusion).
- Clinical metrics measure ICG preparation time (single versus multiple injection of ICG), latency of display (real-time versus non real-time) between ICG and **ActivSight™**, quantification of relative tissue perfusion on clinical decision, specificity of display requiring multiple injection in ICG comparison group, any episode of intraoperative decision change, usability by surgeon and support personnel satisfaction on 5 point Likert scale, and per use and overall cost of each technology.
- Clinical outcome on follow up on postoperative day 28 will be assessed for clinical response following surgery.
- Adverse Events will be documented intraoperatively from beginning of use to completion of surgical procedure.

5.3 ADVERSE EVENT CRITERIA

This study will utilize the NCI Criteria for Adverse Events (CTCAE v.4) and categorized according to the Clavien-Dindo classification of surgical complication.

An adverse event is determined by the investigator or the sponsor to be either "possibly related" "probably related" or “definitely related” to the use of the device. Adverse event is deemed clinically significant based on the medical judgment of the investigator.

5.4 STATISTICAL CONSIDERATIONS

The total of 20 patients will be enrolled; 14 in **ActivSight™** group will initially enroll 6 patients, followed by additional 8 patients. Eight patients clinically matched for demographics and indications will be enrolled in ICG-based NIR-mediated visualization of tissue perfusion and vascularity as a comparison. Stopping rules will be triggered if it is predicted that stringent adverse event rate will be 20% or higher by Day 28 [27,28]

This trial will initially enroll 6 patients followed by additional 8 patients. Stopping rules will be triggered if it is predicted that the toxicity rate will be 20% or higher by Day 28. Individual trigger thresholds are based on the posterior probability that Treatment Limiting Toxicity (TLT) rate exceeds pre-specified rate or target toxicity rate (20%) is greater than 0.90. Namely, let q = Prob(toxicity| data) in the trial which follows a beta distribution with a non-informative beta (.30, .70) prior, we will stop the trial for review if $Pr(q > \Gamma \mid data) > .90$ where Γ is the adverse event rate (20%). Specifically, the trial will be terminated early if [# patients with adverse event rate] / [number of patients] is 2/4, 3/7, 4/11 or 5/15. Note that, for example, the requirement to stop at 2/4 implies that the trial will be stopped at 2/3. The study would also be suspended if there were at least 2 patients in whom to the device technical issues the treatment is not completed [27,28]. However, if the technical obstacles are overcome, the study may resume, pending input of the Medical Safety Monitor Committee.

The initial cohort will provide limited information on adverse events, as even one adverse event would imply a very high upper bound of an exact confidence level about the rate of adverse event. However, with the second cohort if 3 of 14 evaluable patients (~21%) experience an application limiting adverse event, the upper bound of an 85% confidence interval is 0.50; implying a confidence of 85% that there is no worse than a 50% probability that an application limiting adverse event would occur in a given patient. Similar considerations will be applied to the technical feasibility of the application overall. Thus, a sample size of 14 patients is expected with a planned 20% in-evaluable rate.

Adverse events will be summarized descriptively and tabulations on the type, severity, and relationship to application will be performed and any changes of outcomes from baseline on follow up will be examined using the nonparametric Wilcoxon rank test.

Expected outcomes: No major treatment related adverse event intraoperatively.

Non-inferiority or equivalent to ICG-based technology

Non-inferior clinical outcome rate at postoperative day 28 follow up.

6 HUMAN SUBJECTS PROTECTION

6.1 RATIONALE FOR SUBJECT SELECTION

Subject accrual in regard to gender, and racial and ethnic groups is described in Section 1. No groups are being excluded from participation in the trial.

6.2 POTENTIAL PARTICIPATION OF CHILDREN

This trial is designed to define the safety and feasibility of **ActivSight™** in visualizing tissue vascularity and perfusion intraoperatively for any intestinal anastomoses and the inclusion criteria allows all age group. Therefore, children will be entered onto this research trial. Patients who are 18 years old will be offered the opportunity to assign DPA prior to study entry. Adults (18 yrs) who are cognitively impaired prior to study entry and who have not previously assigned DPA to a family member or friend will not be eligible for the trial, because they cannot give informed consent. This trial will be conducted by a team of surgeons, scientists, and engineers who have experience in performing investigational trials in both children and adults.

6.3 RISKS/BENEFITS ANALYSIS

The primary risk to patients participating in this research study is the potential adverse event related to the Laser speckle technology and associated risks with procedure such as general anesthesia. The protocol provides for detailed and careful monitoring of all patients to assess for any adverse event. The primary objective of this trial is to determine safety and feasibility of the **ActivSight™** and non-inferiority to ICG-based visualization technology. The potential benefits from this therapy are visualization of tissue vascularity and perfusion invisible to current practice based on clinical assessment using RGB video

display. Therefore, this protocol involves greater than minimal risk to any participant including children, however, presents the potential for significant direct benefit to participating individual subjects.

6.4 INFORMED CONSENT/ASSENT

The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient or the patient's parents or guardian if he/she is a child, and a signed informed consent document will be obtained prior to entry onto the study. Consent will be obtained by the PI or a research coordinator on this trial. This must be done according to the guidelines provided in the Declaration of Helsinki, ICH E6 Guideline for Good Clinical Practice (GCP), and requirements of Title 21 CFR 50.20 through 50.27. The patient must be made aware and agree that personal information may be scrutinized during audit by competent authorities and properly authorized persons. However, personal information will be treated as strictly confidential and will not be publicly available. Where deemed appropriate by the clinician and the child's parents or guardian, the child will also be included in all discussions about the trial and assent will be obtained when feasible.

7 DATA REPORTING

Unless otherwise stated, all forms will be submitted to:

Peter C W Kim MD
Activ Surgical Inc
840 Summer Street Suite 108
Boston MA 02127
Phone: 617-333-8162
Fax: 617-982-0208
Email: pkim@activsurgical.com

7.1 INFORMED CONSENT

Informed consent should be obtained prior to patient registration.

7.2 PATIENT REGISTRATION FORM

Patient must be registered prior to protocol treatment. The eligibility checklist must be completed and delivered to the study coordinator prior to registration.

7.3 REPORTING REQUIREMENTS

7.3.1 ADVERSE EVENTS DEFINITIONS

Adverse event: An adverse event (AE) is defined as any untoward technical or medical occurrence in a patient or clinical investigation patient administered investigational use of the technology which does

not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal technical function), symptom, or disease temporally associated with the device treatment, whether or not the event is considered causally related to the delivery of therapy.

Such an event can result from therapeutic intervention stipulated in the protocol. Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events only if they result in discontinuation from the study, necessitate therapeutic medical intervention, and/or if the investigator considers them to be adverse events.

Serious adverse event: Any adverse event that results in any of the following outcomes is defined as a serious adverse event:

- DEATH OF PATIENT: An event that results in the death of a patient.
  - Life-Threatening: An event that, in the opinion of the investigator, would have resulted in immediate fatality if medical intervention had not been taken. This does not include an event that would have been fatal if it had occurred in a more severe form.
  - Hospitalization: An event that results in an admission to the hospital for any length of time. This does not include an emergency room visit or admission to an outpatient facility.
  - Prolongation of Hospitalization: An event that occurs while the study patient is hospitalized and prolongs the patient's hospital stay.
  - Congenital Anomaly: An anomaly detected at or after birth or any anomaly that results in fetal loss.
  - Persistent or Significant Disability/Incapacity: An event that results in a condition that substantially interferes with the activities of daily living of a study patient. Disability is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle).
  - Important Medical Event Requiring Medical or Surgical Intervention to Prevent Serious Outcome: An important medical event that, based on medical judgment, may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the patient and may require medical or surgical intervention to prevent any of the outcomes listed above (i.e., death, life-threatening, inpatient hospitalization, prolongation of existing hospitalization, congenital anomaly/birth defect, or persistent or significant disability/incapacity). Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or seizures that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A serious adverse device effect (SAE) is an AE that results in any of the consequences characteristic of a SAE. An unanticipated SAE (USAE) is a SADE which is not anticipated by the risk analysis, while an anticipated SADE (ASADE) is a SADE which is anticipated by the risk analysis.

7.3.2 ADVERSE EVENT SEVERITY

The investigator will rate the severity of each adverse event according to the NCI Common Terminology Criteria for Adverse Events (CTCAE v.4) (See section 5.3) and the Clavien-Dindo classification for surgical complication.

7.3.3 RELATIONSHIP TO APPLICATION

The investigator will use the following definitions to assess the relationship of the adverse event to the therapy:

- DEFINITELY RELATED: An adverse event is temporally related to the use of **ActivSight™**, has been previously associated with the treatment, and no other etiology is identified.
- PROBABLY RELATED: An adverse event has a strong temporal relationship to **ActivSight™** or recurs on re-challenge and another etiology is unlikely or significantly less likely.
- POSSIBLY RELATED: An adverse event has a strong temporal relationship to **ActivSight™** and an alternative etiology is equally or less likely compared to the potential relationship to the treatment.
- UNLIKELY RELATED: An adverse event has little or no temporal relationship to **ActivSight™** and/or a more likely alternative etiology exists.
- NOT RELATED: An adverse event is due to an underlying or concurrent illness or effect of another drug and is not related to the study therapy (e.g., has no temporal relationship to treatment or has a much more likely alternative etiology).

If an investigator's opinion of possibly, unlikely, or not related to **ActivSight™** is given, an alternate etiology must be provided for the adverse event.

7.3.4 ANTICIPATED ADVERSE EVENTS

Since **ActivSight™** does not come in direct touch with patient, any risk of anticipated adverse event is minimal and mostly related to technology failure. The potential risks that may occur during or following the use of **ActivSight™** are:

Classification	Device/procedure relation	Event
minor	**ActivSight™**	Significant decrease in signal intensity of third-party device
minor	**ActivSight™**	Latency in display
minor	**ActivSight™**	Low intensity signal
minor	**ActivSight™**	Temporary blinding discomfort due to speckle intensity
minor	**ActivSight™**	1st degree tissue thermal injury burn
major	**ActivSight™**	Any electro-mechanical issue interfering with OR equipment
major	**ActivSight™**	Revision of anastomoses based on RGB inspection after the use of **ActivSight™**

The table above represents a detailed summary of the risk assessment performed by the sponsor which also includes a summary of outcome and adverse events based on the use of **ActivSight™**.

7.3.5 ADVERSE EVENT COLLECTION PERIOD

Adverse events will be collected and reported if they occur in conjunction with the use of **ActivSight™** and within the subsequent reporting periods. The investigator will immediately (within 24 hours of

awareness) report to the Sponsor all serious adverse events that occur any time after a patient signs the informed consent until a minimum of 28 days after the last therapeutic treatment or until the adverse event has resolved, with or without sequelae, or improved to baseline, the relationship is assessed to be unrelated, patient death, start of new anti-cancer treatment or in the event no further improvement can be expected. The sponsor will review serious adverse events for assessment and determination of continuance of the trial.

Any SAE, including death due to any cause, which occurs to any patient entered into this study up to 28 days following the use of **ActivSight™**, will be reported immediately by the investigator to the IRB and the sponsor as required within all applicable global and local laws and regulations.

Additionally, any SAE that is brought to the attention of one of the investigators after the 28 days following the use of **ActivSight™** that is considered possibly, probably or definitely related to the use will be reported by the investigator to the IRB and to the sponsor as required within all applicable global and local laws and regulations.

All non-serious adverse events occurring from the use of **ActivSight™** until a minimum of 28 days following the last treatment will be collected, whether elicited during scheduled telephone contacts or study visits or spontaneously reported by the patient. Patients will be instructed to report as soon as possible to the investigator or his/her designee any adverse event (serious or non-serious) that occurs during this period. Adverse events will be recorded on the appropriate eCRFs.

7.4 EXPEDITED REPORTING OF ADVERSE EVENTS TO THE IRB

All adverse events limiting the application (Section 3.1.2) with at least a possible attribution to protocol and all serious adverse events that meet criteria (Section 7.3.1), whether related to the use of **ActivSight™**, will be reported to Dr. Peter Kim by telephone within 1 business day of being made aware of the SAE:

Peter Kim, M.D., Ph.D.
Activ Surgical Inc
840 Summer Street Suite 108
Boston MA 02127
Phone: 617-333-8162
Fax: 617-982-0208
Email: pkim@activsurgical.com

If no answer, contact Ms. Claire Nolan, Associate Chief of Staff, at 508-649-4385 who will page Dr. Kim or proxy.

The Protocol PI will report to the local Institutional IRB:

- All serious adverse events (SAEs) that are **not** in the consent form, but are possibly, probably or definitely related to the research. A SAE is defined as an untoward medical occurrence that:
  - resulted in a death;
  - was life-threatening;
  - required or prolonged hospitalization;
  - caused persistent or significant disability/incapacity;
  - resulted in congenital anomalies or birth defects; or
  - required intervention to prevent permanent impairment or death.
  - All other deaths that occur within 28 days of surgery

Reports must be received by the institutional IRB within 7 days of notification of the event.

7.5 CONTINUING REVIEW REPORTING OF ADVERSE EVENTS TO THE IRB

For reporting of adverse events at the time of continuing review, the local institutional IRB requires a summary report of adverse events that have occurred on the protocol since the previous continuing review. The IRB should be provided with the information necessary to clearly identify risks to participants and to make a risk:benefit determination. The summary report is based on the following guidance: any unexpected severity and/or unexpected frequency of expected events need to be reported and interpreted in relation to the risk:benefit of study participants in the narrative.

8 STUDY MONITORING

8.1 GUIDELINES

8.1.1 IRB APPROVAL

IRB approval of this protocol will be obtained from the local institutional IRB prior to subject enrollment. A copy of the initial IRB approval and all yearly continuing review approvals will be maintained. Enrollment will be held if a current approval is not on file with the IRB.

8.1.2 AMENDMENTS AND CONSENTS

Copies of all amendments, consents and approvals will be submitted to the local institutional IRB.

No change to the protocol may be made without the joint agreement of both local instotutional and Activ Surgical Inc. Modifications to the protocol will be prepared in writing, agreed upon by local institution and Activ Surgical Inc., approved by FDA and be submitted to the IRB for approval or notification. Unless the amendment is regarding an urgent safety measure, IRB approval of any protocol changes will be obtained prior to implementation.

8.1.3 PATIENT REGISTRATION

An eligibility criteria checklist will be completed prior to enrollment on this study.

8.1.4 DATA COLLECTION AND REPORTING

The Sponsor will be notified of all adverse events within 7 days and serious adverse events within 24 hours of notification at the participating institution.

8.2 DATA SAFETY MONITORING

The PIs (Local PI and P Kim) will monitor the clinical outcome of each patient treated on the trial and the overall safety and efficacy of the treatment. Local Institutional PI, clinical coordinator, responsible research nurse, data manager, and Activ R & D team will meet on a weekly schedule to review enrollment and observed toxicities.

In addition, and external safety monitor will review any limiting adverse events observed on this trial independently.

8.3 STUDY DISCONTINUATION

The sponsor may discontinue the trial if required to do so by national regulatory bodies, upon IRB request, or due to safety issues. The sponsor shall promptly notify all investigators, IRBs, and the applicable authorities in participating countries should the trial be discontinued or terminated prematurely. Should the trial be terminated prematurely, all treatment related records would be collected by the sponsor. The sponsor will also notify enrolled subjects of this determination.

8.4 USE AND PUBLICATION OF STUDY RESULTS

All unpublished documentation (including the protocol) given to the Investigator is strictly confidential.

8.5 FINANCIAL DISCLOSURE

The Investigator must adhere to regulations regarding financial disclosure in accordance with Title 21 CFR 54.2 to 54.6.

8.6 CONFIDENTIALITY

Research records will be stored in a confidential manner so as to protect the confidentiality of subject information in accordance with institutional policies and HIPAA on subject privacy. The PI and other investigators will not use such data and records for any purpose other than conducting the study. Subjects will be identified on the case report forms by their study number.

The procedures to protect against or minimize potential confidentiality risks include the following: (1) the assignment of unique study subject numbers to patients, (2) the use of these primary identifiers throughout the study, (3) storage of information in locked file cabinets and/or password-protected, encrypted computers, and (4) access limited to study personnel for these file cabinets and electronic data. Only de-identified information will be shared with the sponsor.

Participants are informed that all records are kept confidential. The study will be conducted in compliance with HIPAA requirements. The Principal Investigator and other study staff will assure that the privacy of the subjects, including their personal identity and all personal medical information, will be maintained at all times. Subjects in the study will be identified by a subject ID number to maintain their privacy and confidentiality. Identifying information will be kept in a separate location from data. All data will be identified by study number. Paper records are secured in a locked office. Computer data are protected by passwords, encryption, and file access controls. No identifying information shall be shared outside of the institution.

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