

## **QUESTIONS AND ANSWERS ON EXTENDED DYNAMIC RANGE IMAGING**

### **What is Extended Dynamic Range Imaging (EDRI) and why should I care?**

Extended dynamic range imaging (EDRI) is a proprietary image acquisition technique developed by Loats Associates that enables quality analysis of comet images generated in the single cell gel electrophoresis assay. The EDRI technique overcomes serious limitations with respect to the range of fluorescent light intensities that conventional digital image acquisition systems support. Such limitations frequently lead to erroneous analytic results in the quantitative assessment of comet images. Errors result because the intensity range supported by conventional systems is not sufficient to fully span the wide range of fluorescent intensities commonly exhibited by comet heads and tails and which are important to their analysis. EDRI greatly expands the relative range of light intensities that can be effectively detected and measured, and as a consequence, significantly improves comet DNA distribution and damage assessment capabilities.

### **How are digital images conventionally acquired?**

Fluorescent comet images, visualized with a fluorescent microscope, are generally recorded for quantitative analytic purposes using an electronic camera system, which incorporates a solid state area array sensor chip, formed from light sensitive semiconductor materials. In the typical sensor chip there is a fixed rectangular array of discrete photo-sensor sites onto which images are optically focused and where the corresponding light levels are detected. Each photo-sensor site on the chip is positionally defined by a lithographically formed potential well structure situated just beneath the chip's surface. These potential wells act like tiny attractive buckets where locally generated photo-electrons are collected and held. The chips are frequently cooled to prevent or minimize noise, in the form of thermally generated electrons, from accumulating in the potential wells.

When light radiation is absorbed at or near the surface of the light sensitive semiconductor chip, electrons are generated by means of Einstein's photo-electric effect. The local rate of electron production is proportional to the local intensity of light incident on the chip. Thus, an image, focused on the chip's surface, produces a corresponding pattern of electron generation. And, the generated electrons accumulate over time as charge packets in the underlying regularly distributed array of potential wells.

After a controlled interval of exposure the accumulated charge packets in the wells are electrically transferred into shift registers that are located deep within the chip and protected from any further charge accumulation due to light inputs. The charge packets are then systematically filtered, amplified, and serially read-out by appropriate electronics to produce an electrical signal, whose temporally varying voltage level maps the relative spatially varying intensities of light present in the original image optically focused on to the chip. This signal is then digitized by means of an analog to digital converter to produce a digital (numeric) image, which can be used to support quantitative analytic activities.

Digital images, thus obtained, exhibit a limited dynamic range that must be appropriately matched to the analytic tasks to which they are to be applied.

**What is dynamic range and what limits it?**

Dynamic range is defined as the ratio of the maximum to the minimum detectable (and quantifiable) intensities supported by the system. The upper end of the dynamic range is limited by the charge saturation level of the potential wells in the sensor which collect the photo-generated electrons (i.e., the maximum amount of charge that the buckets can collect and hold without spill-over and loss of charge). The lower end of the dynamic range is set by noise imposed limitations and/or quantization limits of the analog to digital converter used to produce the digital image.

**Don't increases in exposure time improve the dynamic range?**

In general, the finite span of light intensities effectively detected by a system can be shifted, but not relatively changed, by altering the exposure time interval. The process of controlling the exposure or integration time interval used for photo-induced charge accumulation in an electronic light sensor is commonly called gating. Gating in and of itself does not change the dynamic range supported by the system. It simply shifts the bracketed span of measurable intensities up or down. Just as in familiar film based photography, decreasing the exposure time permits the system to support a brighter range of inputs. And, increasing the exposure time shifts the response so that the system supports the detection of a finite range of lower intensity inputs. The relative ratio between maximum and minimum detectable intensity levels, however, is always the same, regardless of the gate time. In other words, the system's inherent dynamic range stays fixed. This means any gain in detection sensitivity achieved at the low intensity end, by use of longer gate times, will result in a corresponding loss of capability to measure inputs at the high intensity end due to over-exposure and saturation.

**What are the implications of a fixed and finite dynamic range for comet analysis?**

Unfortunately, the requirements of comet image analysis generally exceed the dynamic range that can be directly supported by most commercially available image acquisition systems. Differences in the fluorescent intensity levels found in the head and tail regions of comet images are frequently so large that there is no single gate time that yields a digital image that spans the full range of intensities needed for accurate quantification. Gate times, set so that low level comet tail intensities can be properly detected and quantified, result in saturation of brighter head intensities and a consequent loss of information about the amount and distribution of DNA in the head. Conversely, gate times properly set for analysis of bright comet heads frequently result in failures to accurately detect and quantify DNA in the tail. Either condition can result in significant errors in the analytic assessment of comet images, as accurate analysis is critically dependent upon an ability to properly assess the relative distribution of the fluorescently labeled DNA within both the head and tail regions.

At the study level, such analytic errors may result in failures to discern real experimental effects, over or under reporting of group cellular DNA damage, greater overall experimental variability with a corresponding loss of statistical power, and failures to duplicate results in repeated studies.

Fortunately, LAI has overcome this potentially serious problem by developing the EDRI system, which generates images whose radiometric dynamic range characteristics are appropriately matched to the quantitative needs of comet analysis.

### **How does LAI's EDRI system work?**

LAI's EDRI system acquires multiple images of a targeted comet scene, each at a different gate time. The shortest gate time is selected to properly map the brightest features in the scene without saturation, while the longest gate time is selected to best map faint features. The system then combines these multiple images by appropriate mathematical means into a single composite image that has proper relative radiometric scaling and possesses a significantly larger dynamic range than that of any of the individual images from which it was produced. This resultant image provides a greatly improved basis for accurate comet quantification.

The accurate dynamic range extension capabilities of the EDRI system have been demonstrated in serial dilution experiments using suspensions of fluorescent beads, dual label protein gel experiments using both radioactive and fluorescent markers for comparative quantification, and in brightfield densitometric studies using a wide range of neutral density filters.

### **What are the benefits of EDRI?**

EDRI provides high dynamic range images that yield much more stable and reliable analytic results than conventional image acquisition systems can provide. It eliminates a potentially serious source of study error and variability, and consequently improves the statistical power you can get from your comet studies. EDRI enables comet studies to be analyzed with much greater sensitivity, accuracy and repeatability.

If you care about the quality of your comet studies you will want to use EDRI to acquire your comet images. After all, if a study is worth doing, isn't it worth doing right?