Supporting Information:

Buffers – $NaHCO_3$ buffer at a concentration of 0.1M and pH 8.5 was used for the immunfluorescence labelling of the *Giardia* cysts. Fluorescence enhancing buffer (FEB) as reported by Arnaud was prepared by adjusting a 10mM aqueous solution of acetic acid to a pH of 4.7 with 1M sodium hydroxide. To this was added 0.1% Triton X-100 and 50 µM of trioctylphosphine oxide (TOPO). The solution was then filtered through a Microcon 0.2µm filter (Millipore).

Immunofluorescence labelling of Giardia cysts

A 100µl aliquot of *Giardia* cells (*Giardia lamblia* $10^6 ml^{-1}$, (Biotech Frontiers, Sydney, Australia) was transferred to a 1.5 ml eppendorf tube and centrifuged for 45 minutes at 13,000rpm and 4°C. After removal of the supernatant the bead was resuspended using a 200µl aliquot of anti-Giardia G203 antibody (Biotech Frontiers, Sydney, Australia) prepared by 1:100 dilution with $NaHCO_3$ buffer. Incubation was for 45min at RT followed by 45min at 37°C then centrifuged for one hour at 4°C, 13,000rpm. The supernatant again removed and the bead resuspended using a 200µl aliquot of Biotin-anti-mouse antibody (Sigma Antimouse IgG-Fab specific- Biotin antibody; B7151-1ML) at 1:100 dilution with $NaHCO_3$ buffer. A further incubation for 45min at RT, 45min at 37°C followed by a final centrifugation for 1 hour at 4°C, 13,000rpm. The pellet was gently washed with $NaHCO_3$ buffer and the *Giardia* cysts resuspended in 10µl of fluorescence enhancing buffer (FEB) to produce a final concentration of around 500 cysts in 0.5µl.

Conjugation of BHHCT to streptavidin

A 200µl 1:1 mixture of dimethyl formamide (DMF) and $NaHCO_3$ Buffer (0.1M, pH 9.1) was used to dissolve 1 mg of streptavidin (Sigma S4762-10MG). The streptavidin solution was then added to 800µl $NaHCO_3$ Buffer (0.1M, pH 9.1) and vortexed to deliver a clear solution.
The molar absorption coefficient at 330 nm for BHHCT is 3.0×10^4 M^{-1} cm^{-1} and a 1:500 dilution in 0.1M bicarbonate buffer shows an absorbance of 2.09 at 320 nm (eppendorf BioPhotometer) when the BHHCT solution is at the desired strength. Approximately 1.5mg of BHHCT was dissolved in 200µl DMF; target concentration of BHHCT in DMF was 7 $mg.ml^{-1}$ and the desired concentration was reached by addition of extra DMF as determined by monitoring the absorbance value.
The 1 ml solution of streptavidin was magnetically stirred as 100 µl of the BHHCT solution was added to the streptavidin in 10µl increments. After 90 min stirring at room temperature, the solution was transferred to a *Microcon* 0.2µm filter (Millipore) and centrifuged at 4°C, 13,000rpm for 2min to remove particulates. To the filtrate was added 10 µL of 28 mM europium chloride solution.

Streptavidin-BHHCT conjugate purification

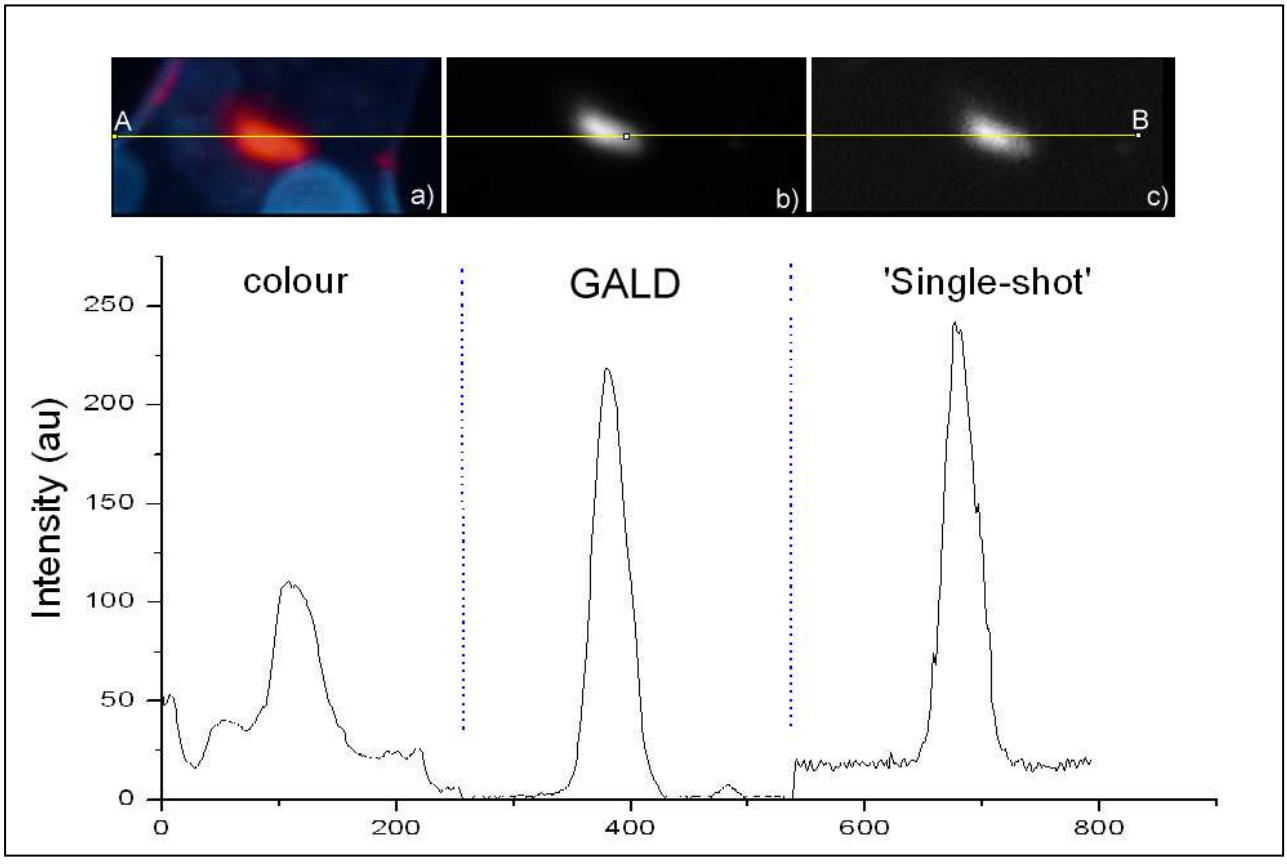
A slurry of 3.5 g Sephadex G-25 Superfine (Sigma Aldrich G25300-50G) in 45ml fluorescence enhancing buffer (FEB) was poured into a 1.5cm x 15cm column. The conjugation product was applied to the column and eluted in 500µl aliquots using FEB. Europium luminescence of the eluate fractions was monitored using a UV lamp to capture the peak fraction containing the streptavidin conjugate. The dilute conjugate solution was then concentrated with Ultrafree Biomax 50KDa filters to a final volume of 50µl. The undiluted BHHCT/streptavidin conjugate (3.5µl) was applied to 0.5µl of pre-biotin-antibody labelled cysts and the mixture incubated for 30 minutes. The labelled cysts were then introduced into the water sample and analysed on an Olympus BX51 microscope for the work reported here.

High speed GALD imaging

Our interests in time-gated luminescence centre on the ability of this technique to reduce image complexity and thereby facilitate automated image analysis. Images without labelled target are completely black and can be discarded, those with prospective targets can be analysed at high speed

with current machine vision systems. Throughput however is limited by frame capture rates and the comparatively long exposure intervals required by the DP72 camera exclude it from high speed imaging applications.

Fig. 7 A *Giardia* cyst labelled with luminescent europium chelate was captured in three different modalities (a) full colour on a DP72 camera (18 s exposure); (b) Monochrome on an Andor iXON camera with the GALD in operation, 100 ms exposure, gain =100 and (c) monochrome on the Andor iXON with 5 ms exposures, gain = 1000, averaged over 5 frames. A line-profile (trajectory shown in yellow) plots signal intensity for each of the modes. Frame (a) was captured in conventional epifluorescence mode, autofluorescence is clearly visible in the line plot with an intensity of 50 at X = 10 to deliver SNR of 2:1. The middle frame (b) shows the labelled cyst with a marked reduction in background, SNR is now 114:1. The rightmost frame was captured at a ten-fold higher gain setting on the iXON camera resulting in significant increase in the background signal; a SNR of 12:1 was recorded in this instance.



For high speed imaging, the BX51 microscope was equipped with an Andor iXON electron amplifying CCD camera (DU-885K-C00). This (-80°C) cooled camera supports SVGA resolution (1024 x 1024) and delivers exceptional sensitivity to enable the image-capture of labelled cysts with a single exposure of 5 ms. The Andor iXON was fitted to the previously reported TGL microscope workstation [30]that was limited to a single 'shot' capture. In this mode, sample luminescence was excited from a single brief pulse of light and the camera triggered 5µs after the excitation pulse had ceased. Frame averaging was used to combine multiple single captures and improve resolution but integration of delayed luminescence over two or more cycles was not possible due to the camera design. With the

GALD however, TGL operation could be achieved without sophisticated gating or triggering of the iXON camera and good quality TGL images were obtained in 100 ms or less. Fig. 7 is a sequence of images of the same labelled *Giardia* cyst captured (a) with the DP72 camera; (b) the GALD/iXON combination and (c) the 'single-shot' mode iXON camera. To help with the analysis a line profile was generated using the pixel values between the points 'A' and 'B' as shown in the lower half of Fig. 7.
The signal-to-noise ratio (SNR) for the colour image shown in Fig.7a was (2.6:1). The GALD captured frame (Fig. 7b) had a superior SNR of (217/1.9 = 114:1) compared to Fig. 7c 'single-shot') frame (238.7/19.6 = 12.2:1) even though five frames had been averaged to generate it. The iXON exposure period with the GALD was 100 ms, corresponding to 62 TGL cycles (1.62 ms per cycle) and was captured at a lower gain setting (100) compared with 'single-shot' mode (1000). The higher gain setting was necessary in single-shot mode to capture an image with satisfactory contrast at the cost of slightly elevated background levels. Fig 7c is also notable in that it has a grainy appearance when compared to Fig. 7b. The difference in image quality is due primarily to the reduced photon count for single-shot mode, mode, it is possible to compensate for faint sample luminescence with the GALD by increasing the exposure period.

Comparison of relative detection efficiency- it is instructive to compare in relative terms, the number of photons reaching the detector when the TGL microscope is operated either with the GALD or in single-shot mode. Assuming a luminescence lifetime for the europium chelate of 640 µs, and equivalent excitation potential of the LEDs for both systems, the proportion of the initial luminescence energy (I_0) emitted by the sample at time-zero (T_0) that is available after the gate-delay interval (T_d), can be roughly calculated. For the europium chelate used here, single exponential decay kinetics ($I_t = I_0\, e^{-td/\tau}$) are known to apply, where τ is the luminescence lifetime (in µs) and 't' is the gate-delay interval (220 µs for the GALD and 5 µs for single-shot mode). The image acquired with the GALD required an exposure of 100 ms which corresponds to 62 full cycles of excitation and detection.

GALD: $I_{260} = 62\, I_0\, e^{-260/640} = 41.3\, I_0$.

Single-shot: $I_5 = I_0\, e^{-5/640} = 0.99\, I_0$

Clearly, photon throughput with the GALD is scalable; simply by increasing the camera exposure period it is possible to integrate multiple excitation cycles. This option is not available in single-shot mode, instead single frames were averaged (5 in this case) to improve image quality but image processing cannot compensate for the greatly reduced photon collection efficiency of this mode. As noted earlier, the excitation efficiency of the GALD with a 40 X objective fitted was more than 7-fold greater, so signal intensity would be correspondingly greater using the GALD. Unlike single-shot **Fig.**

Fig. 8 Data for these plots was sampled by means of a high speed photo-transistor (Epigap, EPD-365-0/1.4) mounted on a metal slide. The device measured the incident energy (arbitrary units) at focus to compare the GALD with a UV LED mounted in a dichroic filter cube [30]. The objective was an Olympus 40X/0.75 Ultraplan (dry); the two pulses are superimposed here for ease of comparison. The GALD pulse has a duration of 374 µs and an average intensity of 3.72 (arbitrary units) whereas the filter-cube mounted UV LED delivered an intensity of 0.512 with a pulse width of 811 µs. Intensity of the GALD pulse was 7.2-fold greater than the filter-cube mounted LED pulse. The

intensity was measured for 10X, 20X and 60 X objectives, respectively the GALD pulse was 3.3, 5.4, and 4-fold more intense for each. The rapid rise and fall times of the pulses shown in this plot are due to electronic switching of the LEDs, passively switched rise and fall-times as shown in the inset were much longer at 69.53 µs and 72.72 µs respectively.