Overcoming crosstalk in luminescence images of mineral grains

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ABSTRACT

Luminescence imaging systems are becoming available for use in luminescence dating, and could potentially allow the dating of sediment and rock at a microscopic scale. For this to be achieved, analytical methods must be developed for turning the data-rich images into reproducible luminescence signals. At present, luminescence signals are collected from images after identifying Regions of Interest (ROIs) — a group of pixels mapped to a luminescent region or grain; the sum of the net ROI signal provides the measure of luminescence for each grain. However, the design of luminescence imaging systems requires a trade-off between signal focus and signal intensity. To maximise signal intensity, commercial systems use a lens combination which also induces optical aberrations, affecting the focus of the image. The variable focus of the image, combined with sample movement between measurements, means that the ROI signals may suffer from reproducibility problems and that signal crosstalk is a significant problem. Instead, the images should be parameterised so that the inherent signal from each grain can be decontaminated from nuisance factors. We describe a data reduction method which uses a Bayesian hierarchical model to resolve the signal from each grain, with input from an incrementally expanding ROI. When tested with an artificial mixed population of grains, the method is better at recovering the known doses than the standard ROI approach, and has significant potential if combined with optimised measurement systems and pre-processing software.

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1. Introduction

Luminescence dating of sediments has been a key chronometric technique with diverse applications in archaeology and palaeoenvironmental science. Significant interest has also focused upon accurate dating of consolidated samples, such as burned flints (Richter et al., 2014; Schmidt et al., 2015) or stone surfaces (Greilich et al., 2005; Sohbati et al., 2012). Luminescence dating broadly depends on the measurement of two quantities: the equivalent dose \( D_e \), representing the amount of radiation absorbed by the mineral grains during the burial period; and the dose rate \( D_r \). The burial age is then defined by \( \text{Age (ka)} = \frac{D_e \, (\text{Gy})}{D_r \, (\text{Gy ka}^{-1})} \). In almost all applications of thermally or Optically Stimulated Luminescence (OSL) dating, the measurement of these quantities follows the disaggregation of the sample. For sediment, mineral grains of interest (either quartz or feldspar) are first isolated through sieving and chemical treatments, yielding a homogeneous grain extract. Rock slices are usually crushed. Optical stimulation is provided either by a cluster of LEDs, in which case the grains are prepared in aliquots of tens to hundreds of grains; or by targeted laser on a grain-by-grain basis, with grains placed in a matrix of holes drilled into the sample carrier (Bøtter-Jensen et al., 2000). Estimates of \( D_r \) come from the measured concentration of radionuclides, always performed on a homogenised, bulk sample.

However, the use of bulk estimates for \( D_e \) and \( D_r \) obscures many complexities in the sediment matrix and radiation field.
Complexities arise through a combination of dose-rate heterogeneity, poor bleaching and sediment mixing. Both sediment grains and crystals fixed within stone slices are affected by complex dose fields, due to inhomogeneous distribution of particular minerals (e.g. K-feldspars, or heavy minerals such as zircons), or particular micro-horizons (e.g. ash lenses, heavy mineral lags). Measurements that ignore spatial complexity may lead to erroneous estimates of the sample age (Martin et al., 2015).

One route through these difficulties would be to measure both the luminescence and beta dose rates on intact samples, i.e. rock slices or resin-impregnated sediment. With this approach, spatially resolved estimates of both $D_e$ and $D_i$ could be paired in the age equation, leading to age estimates at the sub-millimetre scale. In principle, this approach would make it easier to identify intrusive or poorly bleached grains, and increase the precision and accuracy of the final age estimate. Recent developments in instrumentation and software have made this goal feasible. Spatially resolved estimates of dose rate can be obtained through a combination of 3D scanning and radiation modelling (Martin et al., 2015), or more directly by placing a sediment slice on a pixelated semiconductor detector (Romanyukha et al., 2017). Similarly, technological advances allowing the production of commercially available luminescence imaging systems have only recently occurred. The first purpose-built devices, which used either film cameras (Hashimoto et al., 1986; Malik et al., 1973; Walton and Debenham, 1980) or microchannel plates (Burgraaf and Haskell, 1994) to provide spatial resolution, were superseded by the development of scientific cameras using charged coupled devices (CCDs). CCD-based imaging provided a significant technological breakthrough by allowing the relatively fast and simple collection and digitization of images, therefore much more complex data measurement and handling procedures could be developed (Howell, 2006). CCD-based cameras have been utilized by a number of luminescence laboratories, but signal intensity limitations have again limited the research primarily to luminescence emissions in the visible band (Baril, 2004; Greilich and Wagner, 2006; Spooner, 2000) albeit with a few exceptions (Duller et al., 1997; McCulloch et al., 2011). More recently, further developments in CCD architecture, such as the creation of electron multiplying registers (EM-CCD chips) to improve low intensity imaging, and the use of back-thinned CCDs and better phosphor coatings to improve imaging efficiency in the UV region, have yielded systems that can reliably detect low intensity luminescence emissions (Chauhan et al., 2014; Clark-Balzan and Schwenninger, 2012). These have now been incorporated into automated luminescence readers available from major manufacturers (Kook et al., 2015; Richter et al., 2013).

Quantitative extraction of $D_e$s from images, however, has quickly found some significant issues (Greilich et al., 2015; Gribenski et al., 2015). For luminescence images to be used for routine $D_e$ determination, the information content of the images must be reduced into luminescence signals. For a resin-impregnated sediment, we may wish these to correspond with the luminescence emissions from single grains (analogous to current single grain dating strategies). Rock slices may require a more nuanced approach in which surfaces of homogenous $D_e$ are identified and pooled for calculation (see Greilich et al., 2005). In each case, the key first step involves appropriately assigning the luminescence emissions that arise from specific areas of the sample in order to construct the dose-response curves of the Single Aliquot Regenerative-dose (SAR) protocol (Murray and Wintle, 2000). The data reduction procedure, as currently implemented, has the following steps:

- Correcting image defects caused by cosmic and gamma ray interactions.
- Defining Regions of Interest (ROIs: a group of pixels for each grain) using a reflected light image taken after each luminescence readout.
- Making a correction for displacement of the sample carrier between images.
- Assigning a signal for each grain using the motion-corrected pixels in each ROI.

This ROI approach to data reduction has been implemented for grains scattered on a sample disc (Greilich et al., 2015), and for grains fixed in micro holes (Kook et al., 2015), but has severe limitations when considering intact samples. The signal collected in ROIs is not simply a function of the light emitted from that grain, but is also influenced by the image quality and the crosstalk from other grains. Commercial imaging systems use a set of lenses to focus the light from the sample towards the sensor, and imperfections in the lens system (spherical aberrations and astigmatism) reduce the quality of focus achieved at the detector position. Focussing can be improved by reducing the aperture, but with a cost in signal intensity. The imperfect focus of the luminescence image creates two practical issues with ROI data reduction:

1. **Variability of focus and sample motion.** The focus quality can vary across the image, with typically poorer focussing towards the edge. If the sample carrier is able to move between measurements, then the focus achieved for any one grain will also vary between measurements. An ROI, even if corrected for sample motion, will collect different light signals depending on the position of the grain.

2. **Crosstalk.** With imperfect focussing, the light signal collected from an ROI may contain a contribution from nearby grains. With variable focussing across the image, the degree of crosstalk is also dependent on grain position, which will change between measurements because of sample motion.

This presents a problem for ROI-based data reduction: even with successful image-segmentation and motion-correction algorithms, the inferred signal is sensitive to the grain position and to crosstalk, which must affect signal reproducibility. Crosstalk can be reduced (but not eliminated) by ensuring that grains are spaced apart–either by careful placement on sample discs (Gribenski et al., 2015), or using the single-grain sample discs designed for the Risø XY laser system (Thomsen et al., 2015). However, one of the potential benefits of imaging systems lies in the measurement of still-intact samples, for which grain spacing is not possible, and for which a means of overcoming crosstalk is required.

This paper seeks a way of defining a grain’s luminescence signal that is robust in the presence of variable focusing and independent of crosstalk. A Bayesian hierarchical model has been developed to describe the OSL signal, so that each grain’s OSL emission can be disentangled from focus and crosstalk effects. The method is tested by trying to recover a known dose, with grains deliberately separated to simplify analysis. It is hoped the approach will permit future dose evaluation on intact samples.

2. **Methods**

2.1. **Equipment, protocol, and image processing**

Measurements were performed with a Freiberg Instruments Lexsyg Research (Richter et al., 2013), a luminescence reader with an automated detector changer and two filter wheels. Optical stimulation was provided by 458 nm (blue) LEDs, with power of 100 mW cm$^{-2}$ at the sample position. Luminescence and reflected light images were detected with a Princeton Instruments
ProEM512B EM-CCD camera, cooled to –70 °C, with avalanche gain (electron multiplication factor) of 30. Detection filters for OSL measurements combined a 2.5 mm Hoya U340 and a Delta BP 365/50 interference filter. Reflected light images used 405 nm (violet) laser diodes and smaller aperture of 4 mm. A\(^{39}\)Sr/\(^{90}\)Y ring-source delivered beta dose rates of –0.06 Gy s\(^{-1}\). Dose recovery measurements were performed using Riso calibration quartz of grain size 180–212 \(\mu m\), further sensitised by repeated irradiation, heating and bleaching. Three batches of grains were placed in stainless steel sample cups, bleached with blue LEDs, and irradiated with –20, –40 and –50 Gy respectively. The batches given 20 & 50 Gy were then mixed together, leaving two batches (40 Gy, and a mixed 20 & 50 Gy). The grains were transferred to Riso single-grain discs, viewed through an optical microscope, keeping one grain to each hole.

The measurement protocol followed a short SAR cycle: preheat of 220 °C for 10 s; continuous-wave optical stimulation for 10 s at 115 °C, power of 100 mW cm\(^{-2}\); luminescence images were recorded every 0.5 s at 0.6 s intervals (allowing time for pixel readout), for the first 3.5 s of stimulation; and a photo was taken after each OSL step. The first image (0–0.5 s) was used to define the signal, with a background defined by the final two images (2.4–2.9 s and 3.0–3.5 s). A cut heat of 200 °C was used following the test dose. A short SAR sequence used two regenerated doses of (~275 Gy). The grains were transferred to Riso single-grain discs, irradiated and optically stimulated under the same measurement conditions as the dose-recovery measurements. Repeated measurements were made after turning the sample disc. Because

\[ p(\theta|\chi) \propto p(\chi|\theta) \times p(\theta) \quad (3) \]

where \( \theta \) is the set of parameters, and \( \chi \) is the data. In other words, the probability of the model given the data (the posterior) is proportional to the likelihood times the prior.

The likelihood denotes the probability of the data, given the model. In our case, the data comes from the measured luminescence densities of the variable-ROI, so the likelihood is provided by the measured luminescence densities and their uncertainties. The prior term specifies what is known about the parameters without reference to the data, and is implemented in the code in two ways. First, the constraints on \( \delta \) and \( 1/\psi \) are implemented by using positive ordered variable types in Stan (i.e. forcing the model to consider only the solutions where \( \delta > 0 \) and \( 1/\psi > 0 \) with ROI area). However, these model constraints are relatively weak, so we include a formal prior for \( \delta \) based on previously measured data. For this, four bright grains were placed widely apart on a single-grain disc, irradiated and optically stimulated under the same measurement conditions as the dose-recovery measurements. Repeated measurements were made after turning the sample disc. Because
the grains were spaced well apart, it can be assumed there is no crosstalk in the variable-ROI signal. The change in luminescence density across the variable-ROI is then a function of ROI size. As \( \delta \) describes the proportion of a grain’s light collected in the ROI, it must lie between zero and one, and increase with the ROI area. For the system used here, a fixed-ROI of ~450 pixels collects about half the emitted light from the grain (\( \delta \approx 0.5 \)).

For a given ROI size, there is variability in the value of \( \delta \) across different measurements of the same 4 grains, reflecting the variability in focus across the image. In Fig. 1b, the data for \( \delta (j = 2, \text{ROI area} \approx 370 \text{ pixels}) \) is plotted as a function of X-Y image location. Plotted this way, \( \delta \) provides a quantitative measure of focus. Higher \( \delta \) corresponds to sharper focus, because more of a grain’s light is collected in the ROI. For our system, focussing is best towards the middle of the image, and worse towards the edge. However, the focal optimum is not located at the centre of the image, and when considered with disc movement, illustrates the desirability of correcting for focus inhomogeneity. Between measurements, the disc is unavoidably displaced, causing grains to be imaged in different locations in each OSL step. The main movement is rotational, which induces focus changes due to the non-centred focal point. In addition, a small translational motion occurs, because the disc is smaller than the disc holder and is conveyed within the instrument on a mechanical arm.

The analysis was performed on a desktop computer running CmdStan 2.9.0. The luminescence signal for each grain is defined by posterior \( \alpha \): the mean and standard deviation of 200 posterior draws provide the central value and uncertainty, respectively. Some examples of the model results are illustrated in Fig. 2. For bright grains (Fig. 2a), the luminescence density rapidly decreases as the ROI size increases. The modelled signal is dominated by the internally derived luminescence, so crosstalk is not likely to be a problem (but the focus may still change as the sample disc is moved). Dim grains (Fig. 2b) may show an initial decrease in luminescence density as the ROI size increases, but there is a significant contribution from surrounding grains that increases with ROI size. Fig. 2c and d show grains of intermediate brightness, which are increasingly susceptible to crosstalk as the ROI size grows.

3. Results

3.1. Single population

Two single-grains discs were prepared with quartz grains previously irradiated to 38.8 Gy. After measurement and pre-processing (cosmic ray removal, identifying hole positions), the two alternative data reduction strategies were used:

1. A fixed, circular ROI with a radius of 12 pixels (~450 μm on the sample disc) centred on the coordinates of each grain-containing hole.
2. A variable ROI at the same coordinates, with incrementally increasing radius.

For simplicity, the signal uncertainties followed Poisson counting statistics (c.f. Kook et al. (2015)). For the fixed ROI, the net signal was used to construct the dose response curve and estimate \( D_e \). For the variable ROIs, the net signals were used to estimate \( \alpha \) using the Bayesian hierarchical model, with the posterior \( \alpha \) used to construct the dose response curve. Due to low sensitivity of the measurement system, only the brightest 25% of grains are used for the data analysis shown here (75/300 grains). Grain brightness was estimated using the first test-dose OSL signal, as judged by each alternative method, meaning that the exact grain selection may vary slightly between methods. This is deliberate, as one of the potential benefits of the variable-ROI method is to distinguish between large signals that result from bright grains, and large signals that result from crosstalk.

Dose recovery results are illustrated in Fig. 3. For the fixed ROI (Fig. 3a) the central-age dose recovery ratio is 0.92 ± 0.03, with overdispersion of 19 ± 2%. For the variable ROI, the dose recovery ratio is 0.97 ± 0.03, with overdispersion of 16 ± 3%. The variable-ROI \( D_e \) is more spread, with relative standard deviation of 29%, compared with 20% for the fixed ROI, but the larger uncertainties in the variable-ROI \( D_e \) cause its overdispersion to be smaller. However, the uncertainties on the variable-ROI \( D_e \) s are not uniformly larger.
than their counterparts in the fixed ROI. The extra uncertainty in $D_e$ comes from the freedom of the model to choose which values its parameters may take, and this freedom changes depending on factors such as focussing and crosstalk. As such, there is a weighting in the variable-ROI $D_e$ towards grains which have precise model fits, and this is subtly different to weighting by grain brightness. In consequence, the central age model is less sensitive to outliers and the dose recovery is improved.

3.2. Mixed population

Five single-grain discs were prepared with quartz grains which...
had been dosed with either 20 Gy or 50 Gy. The mixing occurred before placement on the discs, so it is not known which dose any one grain received. The resulting $D_e$ distribution is shown in Fig. 4, using the fixed-ROI (Fig. 4a) or variable-ROI (Fig. 4b) analytical strategy, showing the brightest 125/500 grains. Ideally, the $D_e$ should correspond to a mixture of two normal populations. Due to crosstalk, however, we can expect some or all grains to indicate an intermediate $D_e$.

To resolve the populations, we created a normal mixture model of two components, using hierarchical Bayesian principles, and coded in Stan. Each component is parameterised by the mean $\mu$, and relative standard deviation $\tau$; thus $\tau$ is functionally equivalent to the overdispersion parameter in the central age model. Like $\mu$, $\tau$ is estimated with the model, and does not need to be specified. However, we insert a weakly informative prior for $\tau$ of $0.15 \pm 0.05$, which covers the range of overdispersion found in the single-population dose recovery.

Population estimates are given in Table 1, with posterior $\mu$ plotted as a histogram in Fig. 4a and b. For the fixed-ROI data, the model resolves the population means with reasonable precision, but with a bias towards the mean of the $D_e$ distribution. This is most evident for the higher-dose population, which underestimates the given dose by ~10%. The dose populations are better resolved from the variable-ROI data (Fig. 4b); the mixture model identifies the population means more accurately, and with better precision (Table 1).

4. Discussion

The use of EM-CCD images of luminescence for single-grain

![Fig. 3. Recovered $D_e$ distributions using the (a) fixed ROI and (b) variable-ROI model, showing the brightest 75 out of 300 grains. Grains received a dose of 38 Gy in a sample cup before being transferred to single-grain discs for the SAR measurement cycle. The $D_e$ distributions are shown by kernel density (KDF) and probability density (PDF) functions. The shaded kernel indicates the best estimate for the recovered dose, using the Central Age Model (Galbraith et al., 1999).](image)

![Fig. 4. $D_e$ distributions and dose estimates for mixed population of grains given either 20 or 50 Gy, showing the brightest 125 out of 500 measured grains. Data analysis followed (a) fixed-ROI method, and (b) variable-ROI model. The $D_e$ distributions are shown by kernel density (KDF) and probability density (PDF) functions. Given doses are plotted as vertical dashed lines. Recovered doses were estimated from the $D_e$ distributions using a Bayesian normal mixture model (section 3.2); the posterior estimates of the populations means ($\mu_1$ and $\mu_2$) are shown as histograms and in Table 1.](image)

<table>
<thead>
<tr>
<th>Population</th>
<th>$\mu$ (Gy)</th>
<th>$\tau$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed ROI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population 1</td>
<td>22.4 ± 1.61</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>Population 2</td>
<td>45.8 ± 2.2</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>Variable ROI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population 1</td>
<td>20.2 ± 0.9</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Population 2</td>
<td>49.1 ± 1.9</td>
<td>0.19 ± 0.03</td>
</tr>
</tbody>
</table>
However, the method grains, with the price paid in precision rather than accuracy. A given dose can be recovered using the modelled luminescence signal, with improved accuracy over the fixed-ROI signal. When tested with a mixed population, the variable-ROI method enables the given doses to be recovered more accurately and precisely.

When combined with improved pre-processing algorithms and optimised measuring apparatus, the variable-ROI may provide a route towards routine EM-CCD measurements of luminescence, and perhaps spatially resolved equivalent dose.

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