SLIMER Long Writeup
Version 1.0: *Evidence For Observation of* ^{14}C

Steve Elliott, Christopher Fowler, Mary Kidd, Michael Ronquest, Erik Shaw

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1 Introduction

This document outlines the main findings of the SLIMER experiment.

2 Description of the Apparatus

2.1 Microscope Body

2.2 Lenses

Include numerical apertures

2.3 EM CCD Camera

2.4 Fluorescence System

2.5 Copper Collimators

one has diameter of 1 mm the other 250 microns.

2.6 Source, Scintillator, Collimator (SSC) assembly

In order to be more consistent with data taking, a holder for the source, scintillator and collimator was fabricated out of plastic. This holder permits reproducible alignment of the three, and prevents the collimator from moving when a source is placed. It also prevents the collimator from bending the scintillator out of the focal plane of the microscope.

3 Recommended Procedure For Data Taking

3.1 Power-up procedure

The equipment should be powered up in the following order:

1. Dia Illumination: switch is located on the external control box, however there is an extra set of controls on the microscope body.
2. Motorized Stage: switch is located on the external power supply box.
3. Fluorescence Light (if used): switch is located on the external light source.
4. EMCCD: switch is on the camera body
5. Microscope Power: switch is on the microscope body, in the back and to the left near round accessory plug jacks.
6. Nikon Elements DAQ software: Select Roper Scientific driver. At this point, the operating temperature of the camera should be selected.

Once Elements has started, the camera will begin to cool. This make take some time, so the camera's temperature should be monitored in the camera dialog. Starting and stopping image acquisition (live view is sufficient) will prompt the camera to be polled and its temperature updated. -80C is the lowest *set* temperature that can be achieved, although setting the temperature to -90C will get down roughly to -85C.

The filter wheel position should be checked, as this can impact light collection. To do this, Go to Devices → Manage Devices → Nikon Microscope. The list of installed equipment should include the filter turret. Select a consistent empty filter slot.

3.2 Recommended EMCCD and microscope settings

For consistency, it is essential that the same settings be used for each run. The recommendations are:

Magnification 20x, to improve light collection

EM Gain 200, recommendation from Chris Murphy at Photometrics

BERT Off, as this can be applied at the software level

Electron Conversion Gain 1/6x , seems to produce good results.

Quant-View Off

Set Temperature -90C, although it should be noted that the temperate never gets this low. Lower temperatures appear to produce a cleaner signal.

Actual Temperature -84C or lower.

Readout Speed 5MHz, the lowest possible when running with EM Gain, in order to decrease readout noise

Clear Cycles 2

Exposure Time 200ms, seems to produce good results

Trigger Internal Trigger

Keep Overlapped? Selected

Clear Mode Grayed out by "Keep Overlapped" selection.

Sensor Mode Grayed out by "Keep Overlapped" selection.

Filter Cube For scintillation only counting, no filter cube should be used.

Dia Illumination Confirmed as switched off, as it can produce low levels of light when on.

3.3 Focusing Procedure

When using a calibration source with the scintillator/collimator holder, the following focusing procedure is used:

1. Place the scintillator/collimator holder into the clamp located on the stage. The collimator should be in place.
2. Rotate the correct objective into place.
3. Begin live acquisition in NI Elements.
4. Power up the overhead (technical term?) light, starting with low illumination and slowly increasing until light is seen in the live image.
5. Adjust the fine and course focus knobs on the side of the microscope until dark "dots" can be seen, and appear to be in sharp focus.
6. Once this is done, further adjust the illumination until the image is reasonably bright.
7. Further adjust the fine focus until the hexagonal features on the CsI slide can be seen.

Once the bottom of the CsI slide is in focus, the calibration source may be placed in the collimator.

3.4 Data Taking Settings

Data is taken in Time Lapse Acquisition Mode. Using a macro, data is collected in hour increments, then the output data file name is incremented. Doing so mitigates the amount of data lost in the event of a camera communication issue, as well as permits the resulting data files to be converted into a standard `.tiff` format using the Bio Formats library.

3.5 Fluorescence Measurements

3.6 Camera Calibration

The camera's RapidCal feature is exploited for calibration purposes. The procedure is to open the dark box, and rotate the metallic silver collar around the camera's input port until the LED light comes on. The LED will flash and change color until the RapidCal procedure is complete: at which point the LED will be solid green. This procedure may take some time if starting from a warm state (it must cool down first). It should also be noted that the RapidCal will only activate if the camera is NOT in communication with the Nikon Elements software. The mapping of the LED state is as follows:

- Blinking Orange: cooling
- Blinking Green: calibrating
- Solid Green: calibration complete.

RapidCal should be done once a week.

4 Data Analysis

The analysis code is written in Python, and utilizes a number of external libraries to enable reasonably fast analysis of data. These libraries include NumPy, SciPy, PANDAS as well as PIL. These may all be obtained within the Anaconda Python package.

The DAQ software writes data into Nikon's proprietary data format `nd2`. While the DAQ software can also export the data frame by frame into standard TIFF format, we have identified an open source package called Bio-Formats `http://loci.wisc.edu/software/bio-formats`, part of the Open Microscopy Environment `http://www.openmicroscopy.org/site` that can do the same. As Bio-Formats is written in java, it can be run on WIT, and is thus convenient. One drawback of Bio-Formats is that the conversion program, `bfconvert`, loads the entire nd2 file into memory at once. It also invokes java with a memory limit of 512 MB. The bfconvert command has been modified to use up to 20GB of memory, with the modified command being `bfconvert_very_large`. However, this tool is limited to converting files of 20GB in size or less.

4.1 Locations on WIT

The copy of Anaconda, which includes the python distribution and libraries used in this analysis, can be setup on the Weak Interactions Team cluster WIT by sourcing this file:

```
/proj/Software/Notes/SetupScripts/setup_anaconda_root.sh
```

Note that this will also setup a copy of ROOT which can interact with the newer version of python.

The Bio-Formats conversion tool is located here:

```
/proj/Software/ImageProcessing/bftools/bfconvert
```

while the modified version, able to operate on files up to 20GB in size, is located here:

```
/proj/Software/ImageProcessing/bftools/bfconvert_very_large
```

The simulation, done in RAT, can be found here:

```
/proj/Slimer/SlimerSims/MySims/SimpleGeo
```

The analysis codes can be found here:

```
/home/ronquest/Work/SLIMER
```

and is kept within a `git` repository. This repository can be checked out by doing this:

```
git clone /home/ronquest/Work/SLIMER master
```

4.2 Analysis Chain

The data flow, from data taking to final histograms is:

- Nikon Elements Data Acquisition → a single `.nd2` file per run
- A single `.nd2` file per run → `bfconvert` → N `.tiff` files, 1 per exposure
- Background `.tiff` files → `background_average.py` → a single `.npz` (NumPy Zip archive) file, which contains 2 `ndarray`s representing the mean and variance for each pixel in the EMCCD camera
- Background `.npz` file + data `.tiff` files → `image_analysis.py` → N crunched data (energy, position, etc..) files in ASCII (`.dat`) format as well as HDF5 (`.h5`) format
- N crunched data HDF5 (`.h5`) files → `hdf5_sum.py` → a single summed, crunched data HDF5 file
- A summed, crunched data HDF5 (`.h5`) file → `slimer_ana.py` (cuts defined in python script) → crunched data with cuts in HDF5 (`.h5`) format as well as histograms in ROOT (`.root`) format

The main part of the data reduction takes place within `image_analysis.py`, where clustering and other analysis is performed. This is computationally expensive, however this stage can be done in parallel on wit, with the output concatenated afterward.

5 Useful Radioactive Sources

While the goal of this study is to detect ^{14}C from a PhyloChip, there are a number of useful radioactive sources used in this study.

5.1 ^{14}C High Intensity

This is a thin window source from Eckert Ziegler Isotope Products. The model number is BF-014-MF2. The activity is deposited on a polymeric Membrane, and the window is 0.9 mg/cm^2 aluminized mylar. The active area is 3mm in diameter. The activity of this source was 0.9853 μCi on Nov. 15ht 2012

5.2 ^{14}C Low Intensity

This source is identical to the High Intensity ^{14}C source, except for the activity level, which was 45.18 nCi on Sep. 1 2011.

5.3 ^{14}C Doped Biological Samples

5.4 ^{241}Am Thick Window Source

This source is an encapsulated steel source made by the IAEA. Due to the encapsulation, the α particles are stopped. The strongest line is at 59.54 keV. The strength was 10.51 μCi on Dec. 1 1970.

5.5 ^{241}Am Thin Window Source

This source is a thin window model by Eckert Ziegler. αs will escape from this source, with the most common decay branch producing 5486 keV αs.

5.6 ^{90}Sr Source

This is a legacy source. The main decay produces βs with a mean energy of 195.8 keV and an endpoint of 546 keV. The daughter, ^{90}Y is also unstable and produces βs with a mean energy of 933.7 keV and an endpoint of 2280 keV.

5.7 PhyloChip

Total area of sample region is 1.28 cm x 1.28 cm. Each pixel is 20 x 20 microns.

5.8 In-hand samples

We also have in-hand a slide of 14C acetate deposited in three small regions. 0.8μL of acetate were deposited, with a total activity of 0.07μCi, leading to a concentration of 0.001μCi/gram

6 Simulation

A number of simulations were run in order to understand the resulting energy spectra produced by different radioactive calibration sources. These simulations were run using RAT, and include only the plastic substrate that contains the ^{14}C and the CsI scintillator. Currently, only the energy deposited into the CsI is recorded. Further studies should factor in the scintillation yield as a function of energy for CsI, as well as include the effect of the protective plastic layer on the CsI as well as the effect of the 1mm thick glass slide that the CsI is evaporated upon. For sources other than the ^{14}C calibration sources, the actual source geometry should also be modeled.

7 Optical Model

8 Results

The studies conducted with SLIMER thus far have focused on the background present in the system, attempts to determine the energy scale of the system, and finally attempts to observe ^{14}C decays as well as other low energy events such as ^{241}Am gamma rays.

8.1 Background Study

The sources of background were studied. The possible sources of background in this experiment include:

- EMCCD noise (electronic noise, "dark current", clock induced charge, etc)
- Stray light caused by light leaks
- Actual scintillation events from background radiation

8.1.1 EMCCD Noise

EMCCD noise was studied by collecting data with the EMCCD camera shutter closed. It should be noted that the camera shutter can be rotated closed *without* activating the camera's Quick Calibration feature by performing this step with the Nikon Elements software running and communicating with the camera.

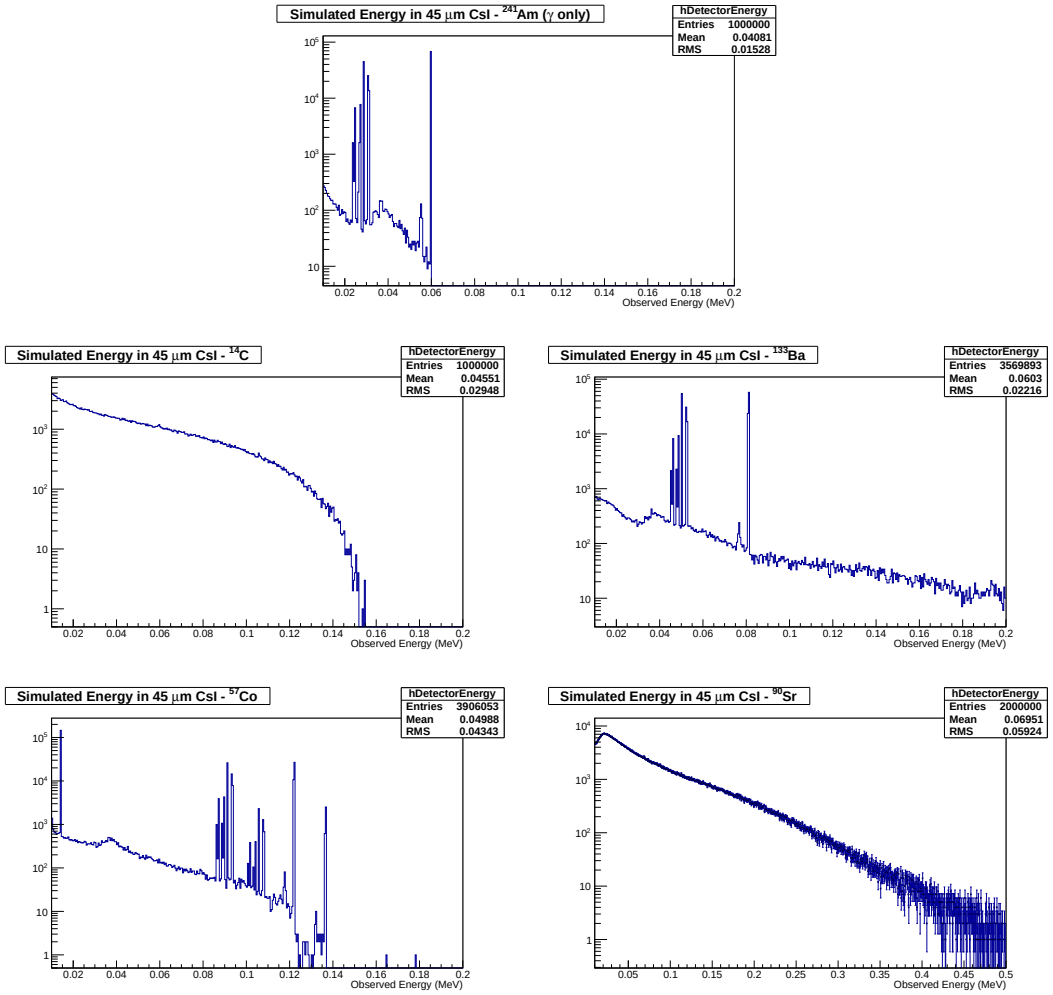

Figure 1: Simulated energy spectra in 45 μm thick CsI for a variety of calibration sources available at LANL

We study the histogram of pixel counts, plotted for every pixel in a image, over a large number of images. The mean value and tail indicate the level of noise present in the EMCCD system. We also study the time dependence of the noise by plotting the mode, median and mean of the pixel counts per image as a function of time. REPEAT THE STUDY, AND ADD THE HISTOGRAM OF PIXEL COUNTS

We also examine how this noise contributes to the actual background by creating "false" events where the noise mimics an actual scintillation event. We run our image analysis code on the above data, attempting to reconstruct scintillation-like events. DO THIS STUDY, AND SHOW THE ENERGY SPECTRUM, NORMALIZED TO TIME, OF THE RESULTING RECONSTRUCTED EVENTS.

8.1.2 Stray Photons

We study stray light caused by light leaks by opening the EMCCD camera shutter, and collecting events *without* the CsI in place. Again, we study the histogram of pixel counts, for every pixel in a image, over a large number of images. The resulting distribution of pixel counts when compared to the same plotted for data collected with the shutter closed reveals the effect due to stray light. We study the time dependence of the light by plotting the mode, median and mean of the pixel counts per image as a function of time. REPEAT THE STUDY, AND ADD THE PLOT We also examine how this stray light contributes to the actual background by creating "false" events where the light mimics an actual scintillation event. We run our image analysis code on the above data, attempting to reconstruct scintillation-like events. DO THIS.

Consider if using the shutter integrated with the filter wheel assembly will tell us anything. If the shutter is located above the filter wheel, just below the object, then collecting data with this shutter closed and comparing it with the shutter open would ID light leaks coming from inside the microscope or outside.

8.1.3 Background Radiation

Finally, we study the effect of background radiation by adding the CsI slide to the system. Again, we study the histogram of pixel counts, for every pixel in a image, over a large number of images. DO THIS, WITH ALL THREE SCINTILLATOR SLIDES.

8.1.4 Summary of Background Studies

ESTIMATE THE MAIN BACKGROUND CONTRIBUTION HERE. THE ID OF THE MAIN SOURCE OF BACKGROUND WILL REVEAL IF WE CAN LOWER THE BACKGROUND.

8.2 14C spectra and efficiency

In order to understand the signature of ^{14}C scintillation in the SLIMER apparatus, we collect data with the two thin window ^{14}C sources in order to achieve high rates relative to background.

8.2.1 High rate ^{14}C source in direct contact with CsI

Data were collected with the high intensity source resting directly atop of the CsI in order to produce the maximum rate of ^{14}C beta interactions in the CsI. The rate was further optimized by manually scanning the telescope across the CsI until the rate of interactions seen with the DAQ software subjectively appeared to be the highest. The magnification of this run was XXXX with an EM gain of XXXX. Data were collected with the source in place, and then an background exposure was done with the source removed.

8.2.2 High rate ^{14}C source in 1.0 mm collimator

Data were collected with the high intensity source resting in the 1mm diameter copper collimator, which in turn was placed within the source, scintillator, collimator (SSC) assembly The rate was NOT optimized by scanning, instead a random point aligned with the collimator hole was selected. The magnification of this run was XXXX with an EM gain of XXXX. Data were collected with the source in place, and then an background exposure was done with the source removed, but the collimator/CsI assembly still in place.

8.2.3 Low rate ^{14}C source in 0.250 mm collimator, edge of collimator imaged

Data were collected with the low intensity source resting in the 0.250 mm diameter copper collimator, which in turn was placed within the CsI/collimator assembly. The rate was NOT optimized by scanning, instead the edge of collimator was imaged. Not only does this permit an estimate of the spatial resolution at low energies, but also provides a final check against the signal: the ^{14}C candidates should reconstruct in the open area of the collimator.

The magnification of this run was XXXX with an EM gain of XXXX. Data were collected with the source in place, and then an background exposure was done with the source removed, but the collimator/CsI assembly still in place.

8.2.4 Discussion of ^{14}C Results

The high rate runs provide our best estimate of the energy spectrum. However a strong extrapolation down to the PhyloChip level will depend on our ability reproduce event rates across this source. Once we have a confident estimate of the ^{14}C AND background spectra, we can project our sensitivity as a function of ^{14}C activity, ^{14}C density and exposure.

We will also want some discussion of the optimization of the setup for ^{14}C counting.

8.3 ^{241}Am (γ only) spectra and efficiency

Data were collected with the sealed ^{241}Am γ source. Here we exploit the 59.6 keV γ line present in the ^{241}Am decay, assume no other lines are present, and note that this source will not permit α particles to escape.

The magnification of this run was XXXX with an EM gain of XXXX. Data were collected with the source in place, and then an background exposure was done with the source removed.

8.4 ^{207}Bi spectra and efficiency

Do we have data in the can to show this?

8.5 ^{241}Am α source

Data were collected with the thin window ^{241}Am α source. Here, we would expect near full energy deposition into the CsI.

The magnification of this run was XXXX with an EM gain of XXXX. Data were collected with the source in place, and then an background exposure was done with the source removed.

WANT TO SHOW ALPHA SENSITIVITY AS A FUNCTION OF EXPOSURE TIME, BACKGROUND RATE AND SOURCE STRENGTH.

9 Observed Problems

Over the past two months, we may have discovered a number of complications in the running of the SLIMER apparatus. These complications should be confirmed and mitigated in the future.

9.1 Light Leaks

A shift in the mode of the pixel count histogram was observed when the camera shutter is opened. This indicates a light leak of some type.

9.2 Communication Problems

The Nikon Elements DAQ software is able to control the filter wheel position. Upon startup, the filter wheel may rotate to a random position. This is a concern as the transmission of light through the two existing filter cubes as well as empty slots can be expected to be quite different and would thus impact the observed energy scale. This can be checked by using the Nikon Element controls, and mitigated by adding this check to the startup

procedure. However, this possibility should be factored in when dealing with older data sets.

9.3 Camera Driver Issues

There appears to be an issue with the Camera Driver, as it sometimes crashes and kills a run. The error message is error "-9".

9.4 Windows Power Management Settings

STOW may be setting the power management options in such a way that renders overnight runs difficult. It is unclear if this problem is due to the monitor shutting off, or the computer being put to sleep.

9.5 Strong temperature dependence

A strong EMCCD temperature dependence is suspected, with lower temperatures results in a higher C14 observation rates. This needs to be checked.

9.6 Focusing Problems

We have seen signs that the focus at the 20x setting changes when the source is placed/removed. Not touching the scintillator slide seems to help, but this may be a weight issue. We confirmed that there is NOT a time-dependent drift in focus. This is mitigated when using the SSC, but will be an issue if bare sample or sources are placed atop the CsI.

9.7 Varying Background Level

We have found evidence for a difference in the variance on the background level (the raw pixel counts) between two background runs taken during different days. The shift in variance is from 7 to 10 counts/pixel. We have also seen evidence of a shift in the mean pixel counts on an image by image basis. This agrees with the observation of a shifting pixel count histogram within the Nikon Elements DAQ software.

9.8 Varying 14C rate

Different 14C event rates have been observed, as well as different end points in the energy spectra, during different runs.

9.9 Optimum Magnification

We have consistently been able to produce good observations at 20x magnification, with few background events satisfying our analysis criteria. However, to check if this magnification

was optimum, we also ran at 10x magnification, with all other DAQ settings the same. For these data, a shift in the energy spectra was observed. While this is expected due to the different numerical apertures of the objective lenses and hence different amounts of light collection, the shift observed may have been larger than expected.

9.10 Decrease in rate due to use of collimator

Even when the field of view is not occluded, we observe a substantial decrease in event rate when the ^{14}C sources are used with a collimator. This is likely due to some combination of effective sample area on the source, solid angle, as well as scattering effects. This needs to be understood, or at least well measured so that we can better estimate the conversion between event rate and source activity.

9.11 Background cluster sizes

We observed that the clustering algorithm would typically find clusters of two different sizes in the background data. One is likely CIC events. Plotting the energy of the cluster versus the size may better separate these two classes of events.

10 To Do List

1. QuantView study
2. High/ Low Intensity ^{14}C spectra.....then scale to expected activity level from samples.
3. Alpha energy + resolution.... assume spread due to photon statistics, estimate light yield. Compare to simulation.
4. Estimate Run time required for phylochip study
5. Collect more data, in particular ^{57}Co, ^{207}Bi, ^{137}Cs and ^{241}Am alphas
6. BERT study, as a function of magnification
7. Scintillation background for all three scintillators
8. Time stability study , requires code
9. CsI uniformity study , requires low magnification.
10. ^{14}C rate study across high rate source ..... need to reproduce our detection rate to extrapolate down the lower rates.
11. Performance/rate as a function of magnification.

12. Fluorescence testing , require proper dyes.
13. Spatial resolution using collimator
14. Code to look at mode and spread of pixel counts per image, as a function of time.
15. Determine low energy threshold of SLIMER.
16. Measure C14 event rate and energy spectrum (as well as background) as a function of magnification, EM gain, conversion gain, and temperate.
17. Do ESR foil study, and estimate the amount of light lost through the top of the CsI.
18. Repeat EMCCD noise study, and add in time dependence
19. Repeat light leak study, add in time dependence and perhaps use filter wheel shutter
20. Repeat background study with the three scintillators.
21. Produce a good sample of images of different classes of events (background, C14, Am241, Sr90, Cs137 and alphas) with the pixels counted as the event highlighted. Both smoothed and unsmoothed data should be displayed, and this should be done at different magnifications.
22. Code to extract camera temperature from frame
23. Code to extract exposure time from frame.
24. Understand if we want to keep overlapped frames
25. Study EMCCD clear mode