Scintillator-Layered Imaging Microscope for Environmental Research

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S.M. Pfiffner, T. Vishnivetskaya, A. Layton, (University of Tennessee)
L.G. Whyte, N. Mykytczuk, J. Allan, R.C. Wilhem, (McGill University)
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P. Hatcher, (Old Dominion University)

LA-UR-11-10260

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SLIMER

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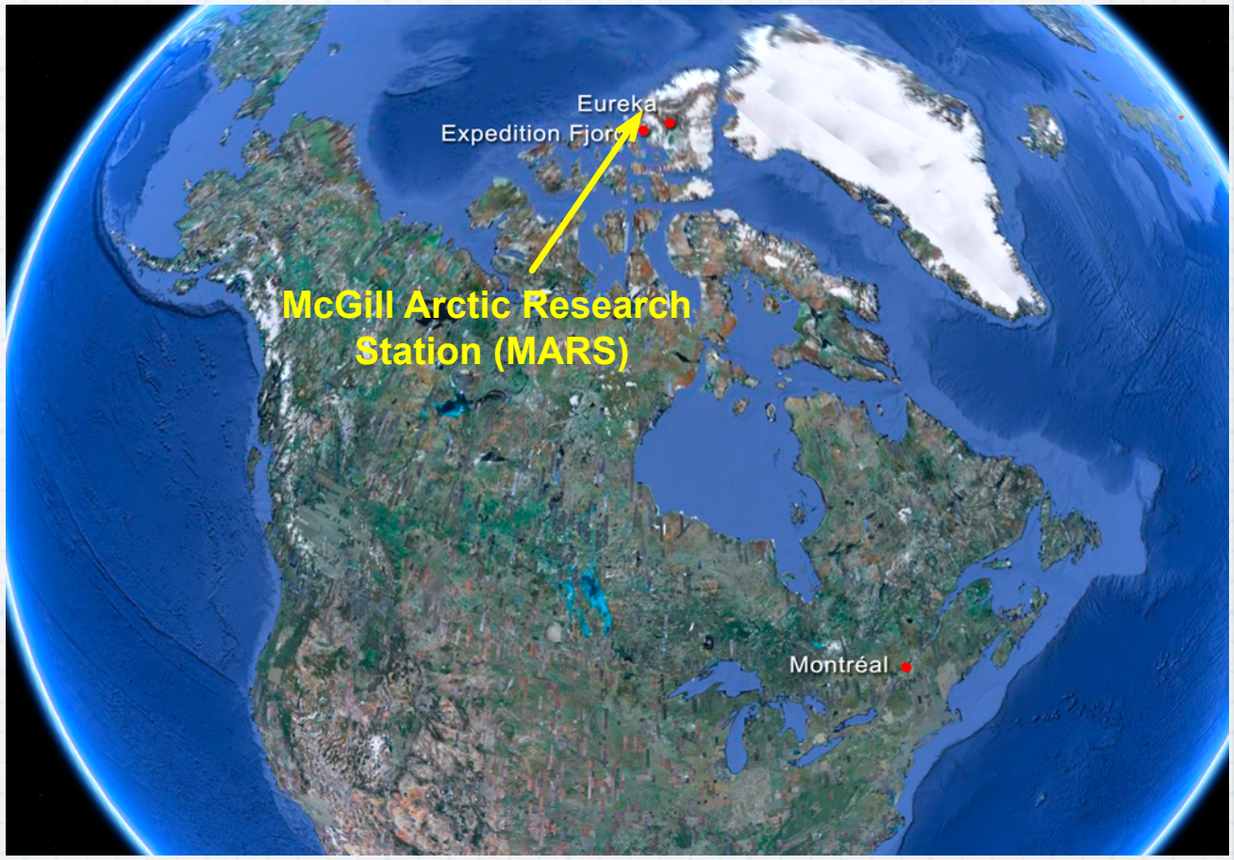
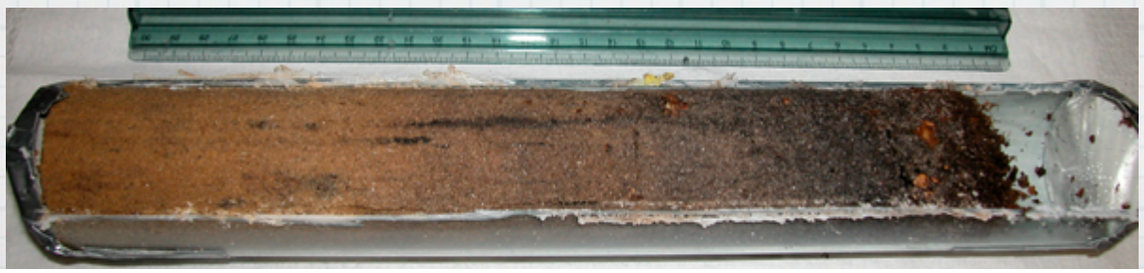
Studying the Permafrost

* Permafrost underlies 22-24% of Earth's surface.
* Global climate models project the strongest future warming in Northern Hemisphere high latitudes.
* Thawing permafrost results in microbial decomposition of previously frozen organic C.

Studying the Permafrost

- We need to understand the rate at which individuals in the microbial community ingest the organic C.
- The microbial community will be fed enriched ^{14}C, which decays via beta decay.
- The microbial community will be divided by type onto a RNA/DNA microarray.
- Each type will be fluorescently tagged.





SLIMER's Objective

* To image fluorescent light from tagged biological samples.
* To detect beta decays from ^{14}C contained within the biological samples.
* **This has never been done in a single step process.**

Previous System - Isotope Array

* Used a beta-imager for radioactivity analysis
* two step process
* limited resolution

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2003, p. 6875–6887 Vol. 69, No. 11
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.11.6875–6887.2003

The Isotope Array, a New Tool That Employs Substrate-Mediated Labeling of rRNA for Determination of Microbial Community Structure and Function

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Received 7 May 2003/Accepted 14 August 2003

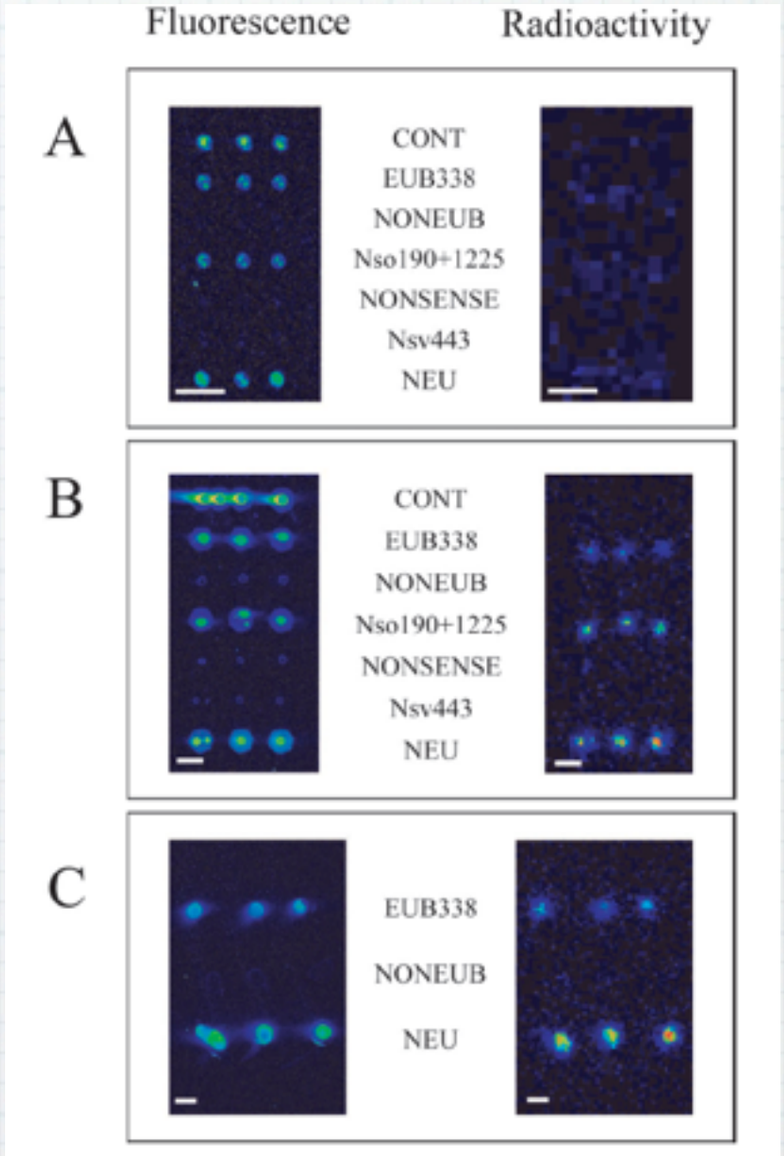
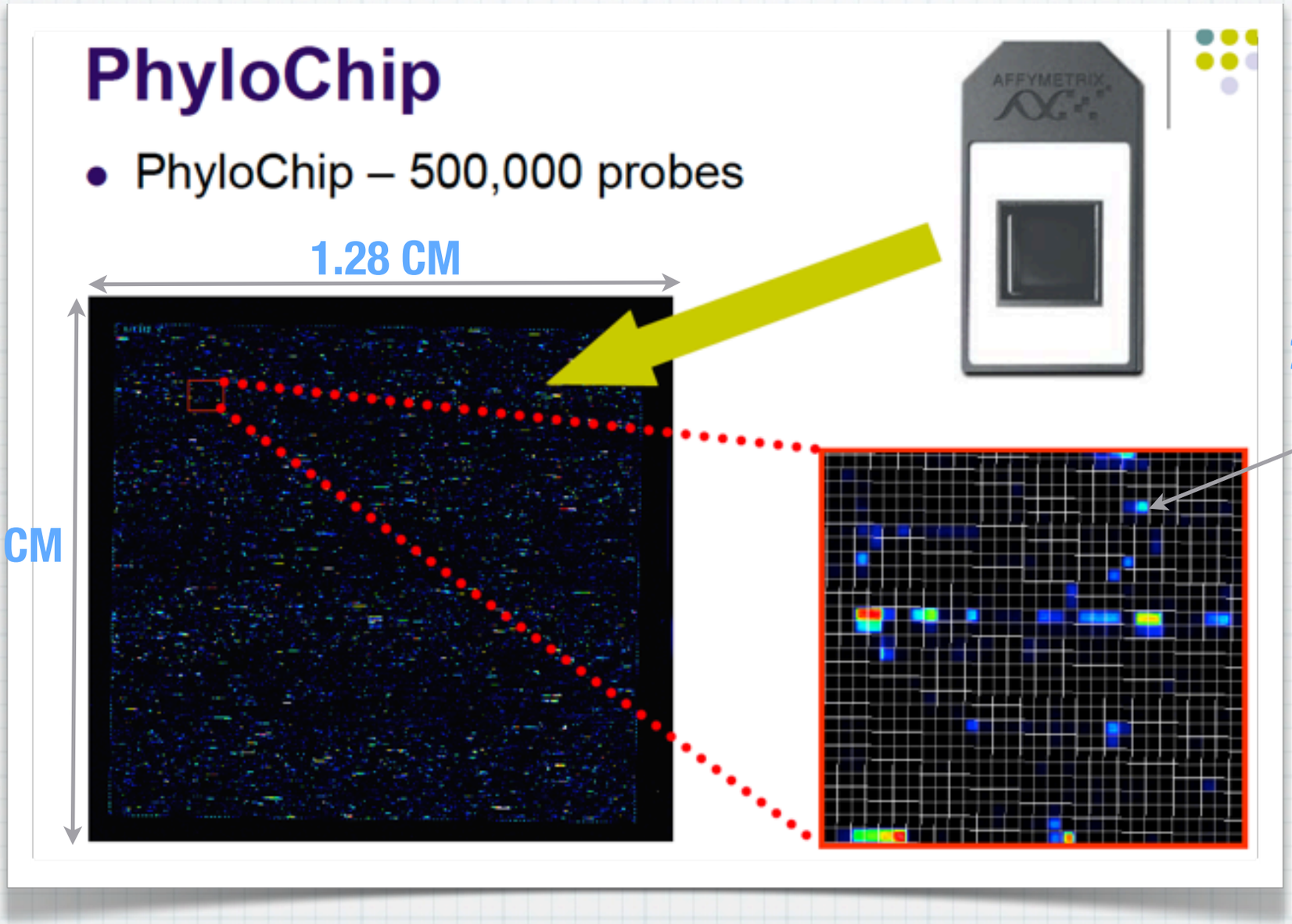


FIG. 2. Isotope array experiment with *N. eutropha* Nm57. rRNA was extracted from *N. eutropha* cultures after incubation with [^{14}C]bicarbonate. After labeling of the rRNA with Cy5, a single slide carrying three prototype arrays with different spot sizes was hybridized, washed, and scanned for fluorescence and radioactivity. (A) Spot diameter, 125 μm. (B) Spot diameter, 500 μm. (C) Approximate spot diameter, 1 mm. Bars, 500 μm.

SLIMER's Objective



SLIMER's Objective

- Idea: overlay biological samples with thin scintillator.
  - electrons from ^{14}C produce light in scintillator
  - fluorescent light passes through
- Therefore, need scintillator with:
  - correct optical properties
  - ability to maintain location information

CsI(Tl)

* Thallium-doped cesium iodide (CsI(Tl)) is a commonly used scintillator in nuclear physics
* Light output is about 65 photons/keV peaked at 565 nm.
* When grown on a substrate with a structure (i.e. fiber optic plate) the CsI(Tl) forms columns which channel the light
* Transmits a large range of wavelengths.

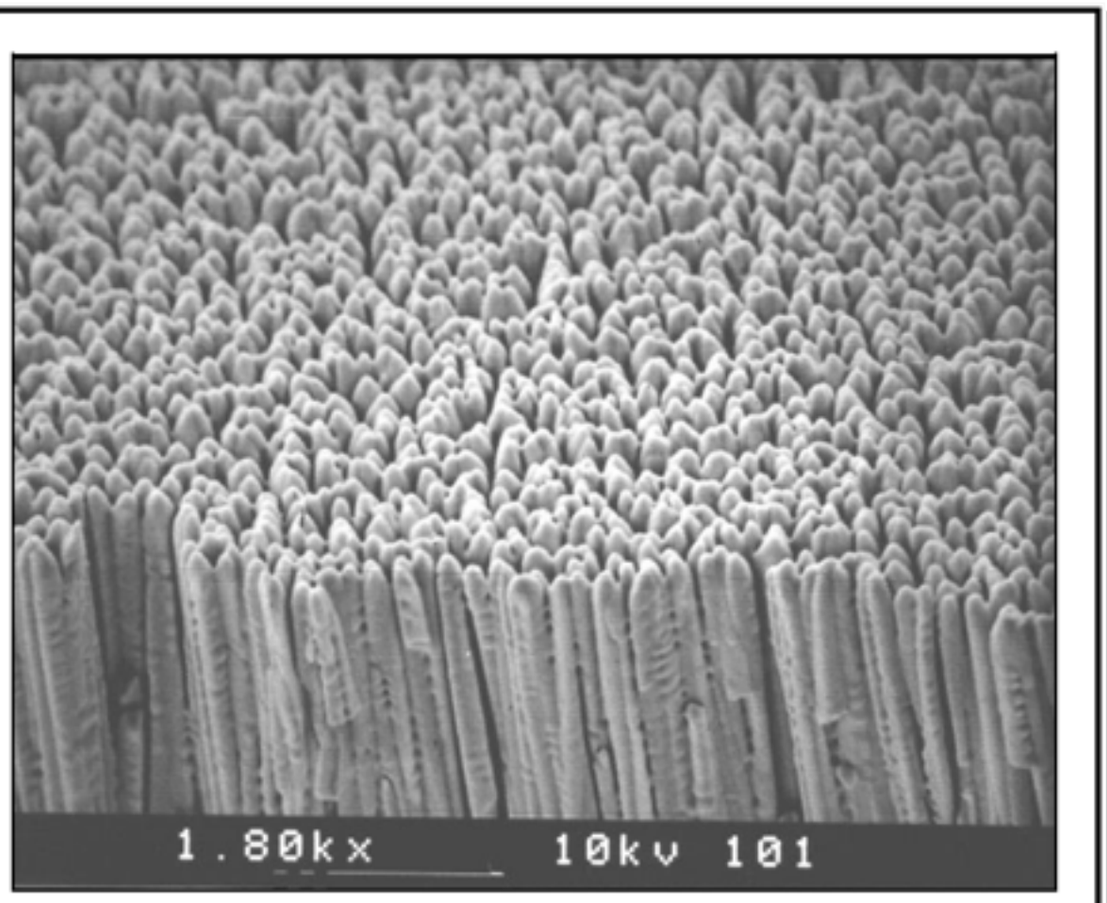
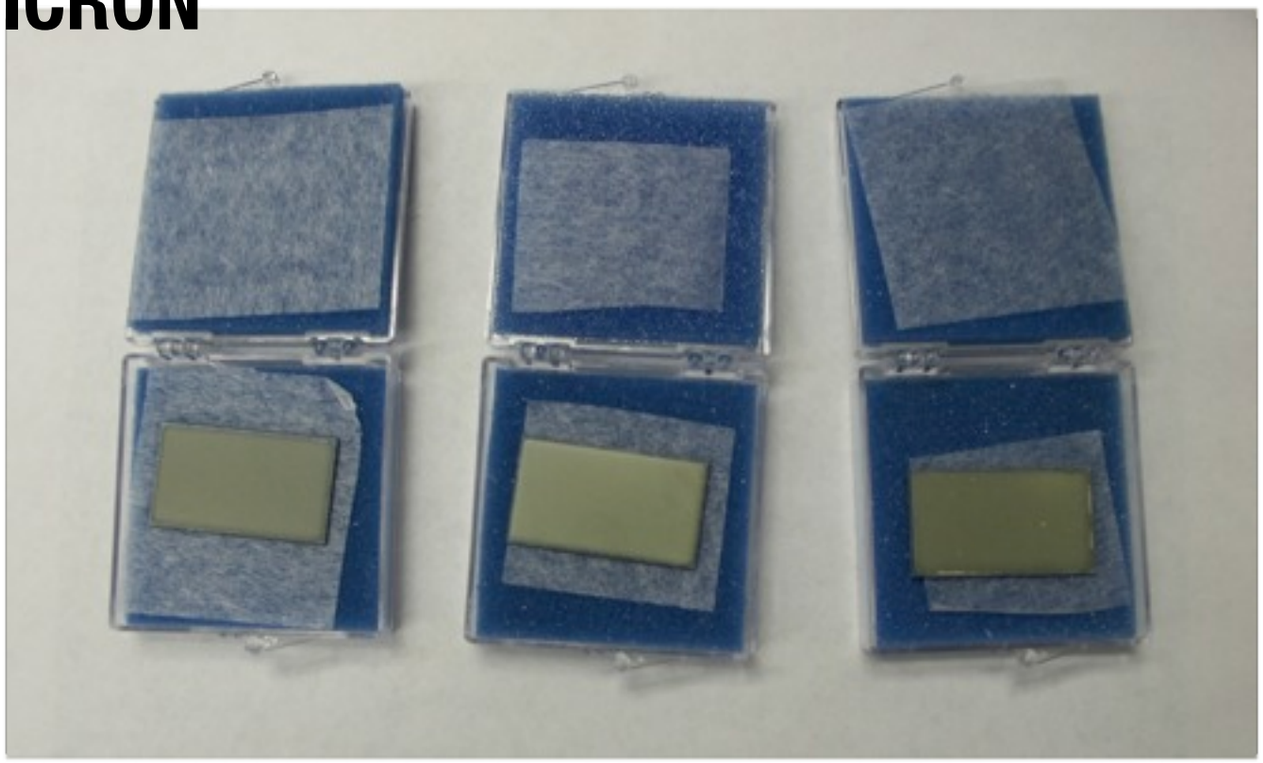


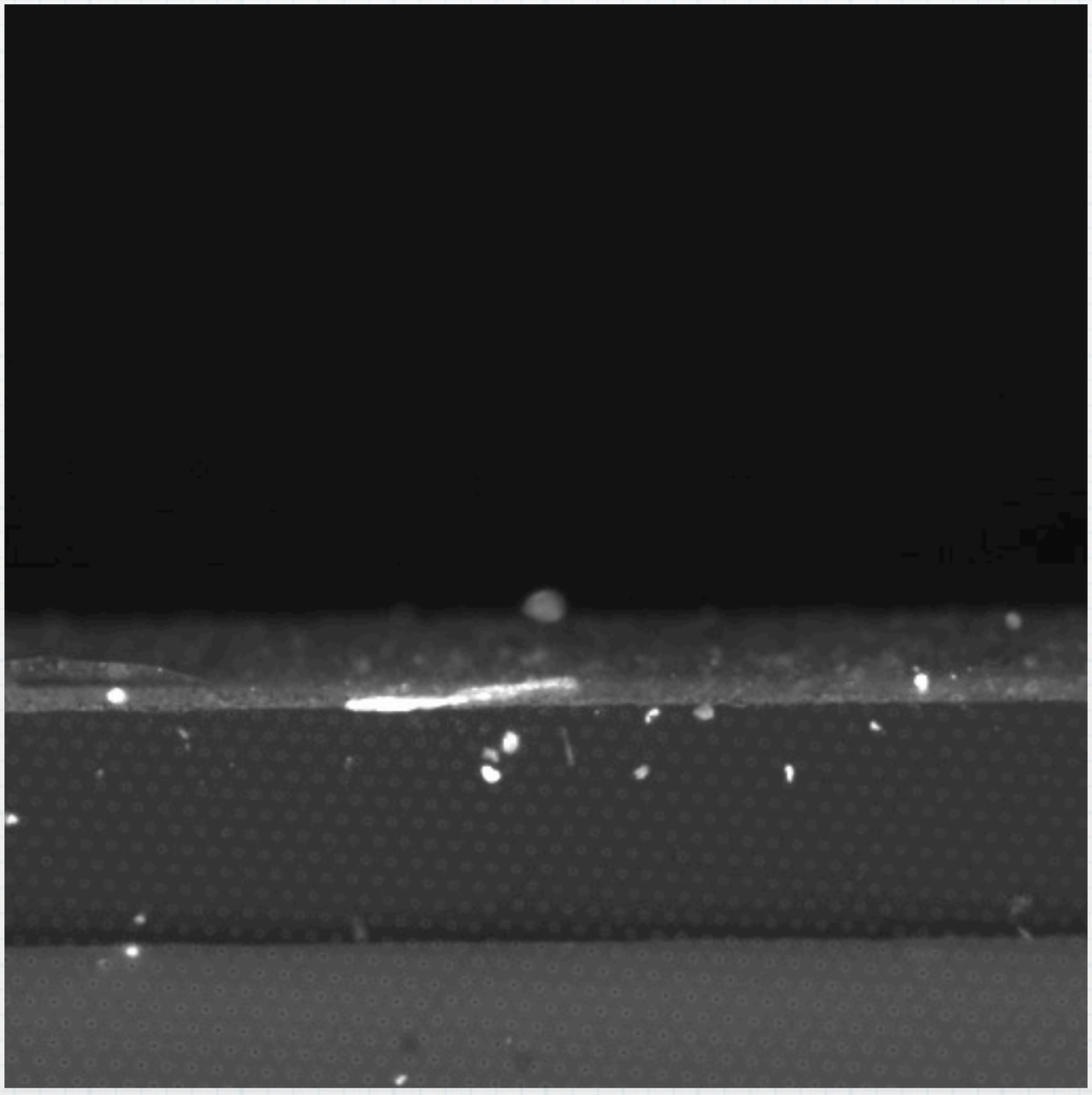
Figure 1. SEM of a 140 μm thick micro-columnar CsI(Tl) film fabricated at RMD

CsI(Tl)


RMD Instruments, LLC
CsI(Tl) Sensor
PN: F1-AT01-12
SN: 12B5324
45 μ
RMD Instruments, LLC
CsI(Tl) Sensor
PN: F1-AT01-12
SN: 12B5414
135 μ
RMD Instruments, LLC
CsI(Tl) Sensor
PN: F1-AT01-12
SN: 12B4924
150 μ
45 MICRON
135 MICRON
150 MICRON

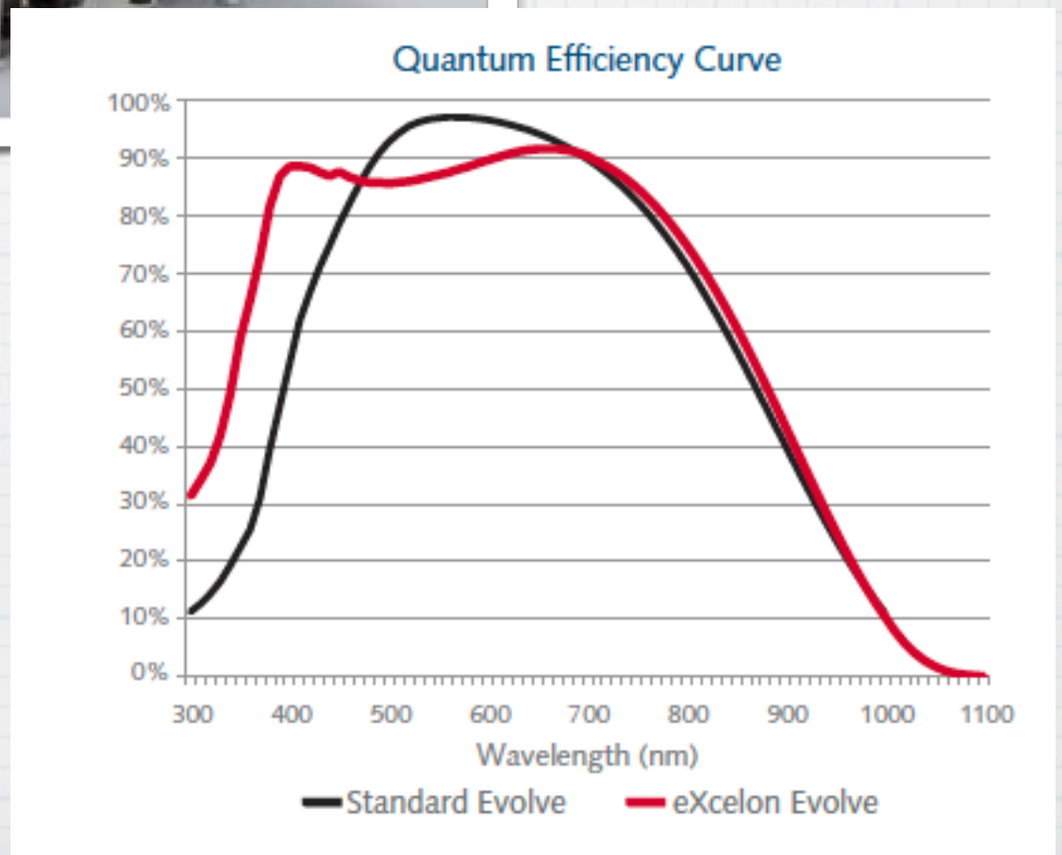


Edge of the CsI(Tl)

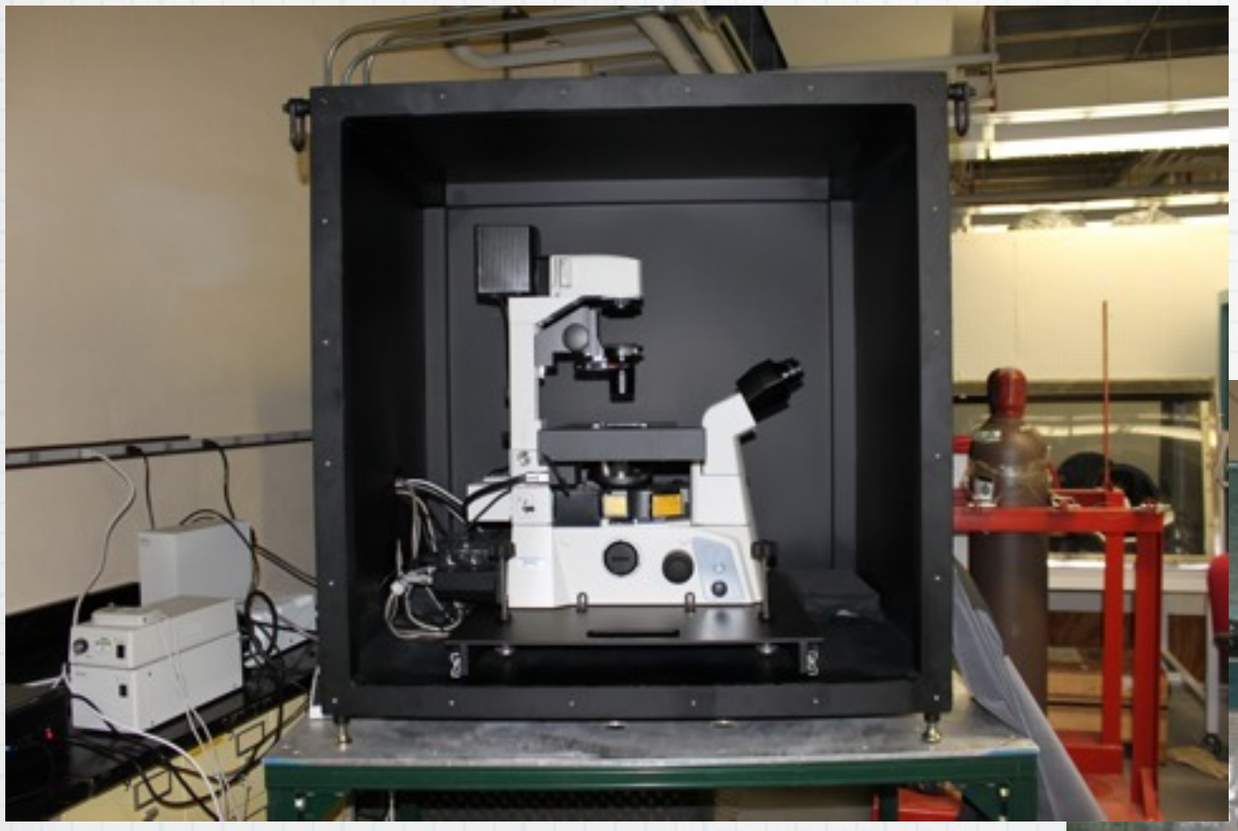


System Components

- Purchased Nikon Eclipse Ti-U.
- Considered transmission of each component:
  - Objectives >85%
  - Filter cube >95%
- Photometrics Evolve- EMCCD
  - on-board EM gain
  - cooled as low as -85 ˚C



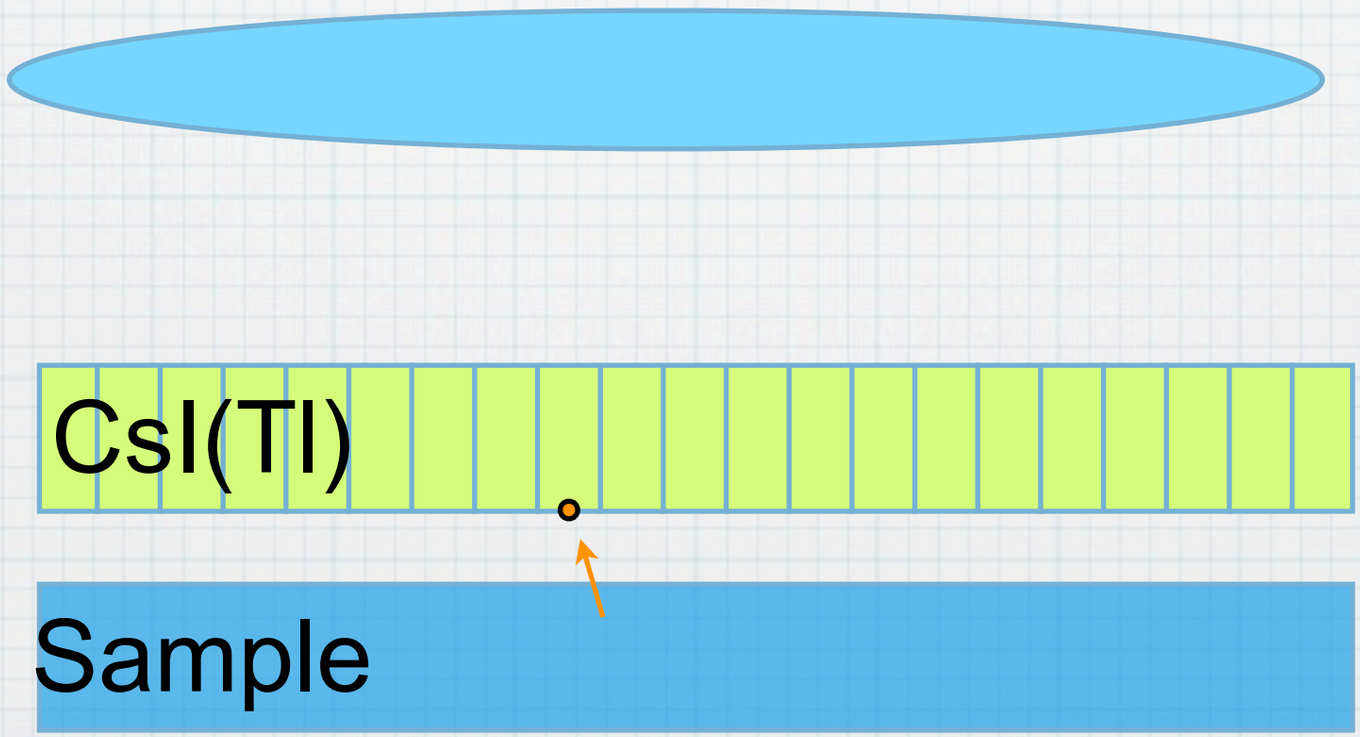
The Setup



Thursday, June 21, 2012

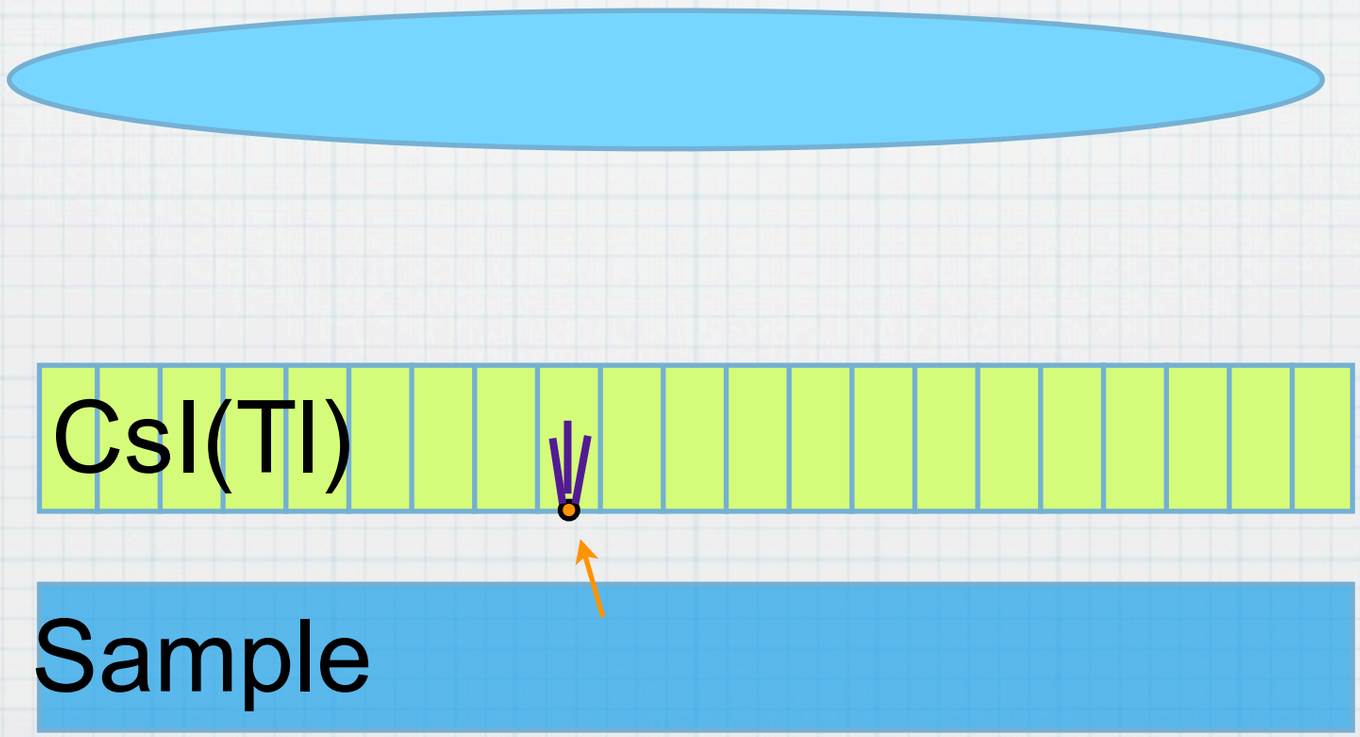
The Signal

EMCCD



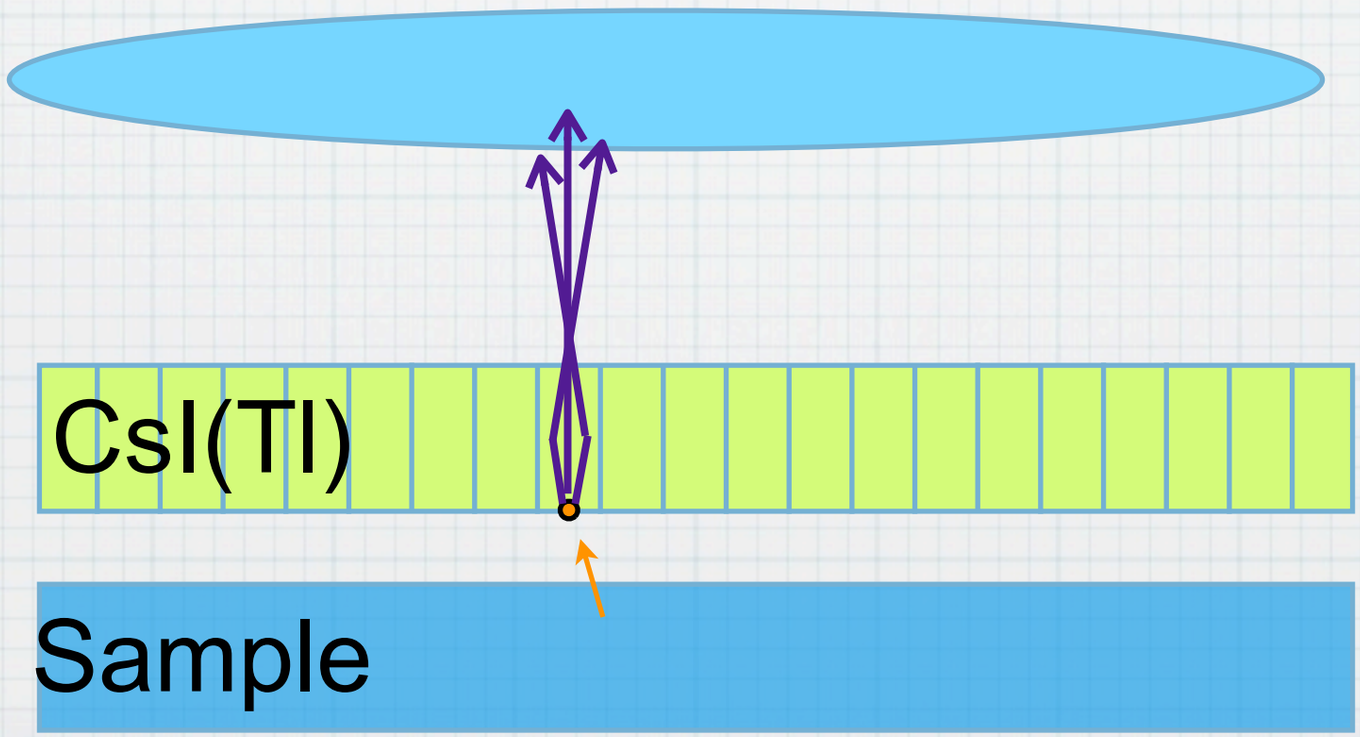
The Signal

EMCCD

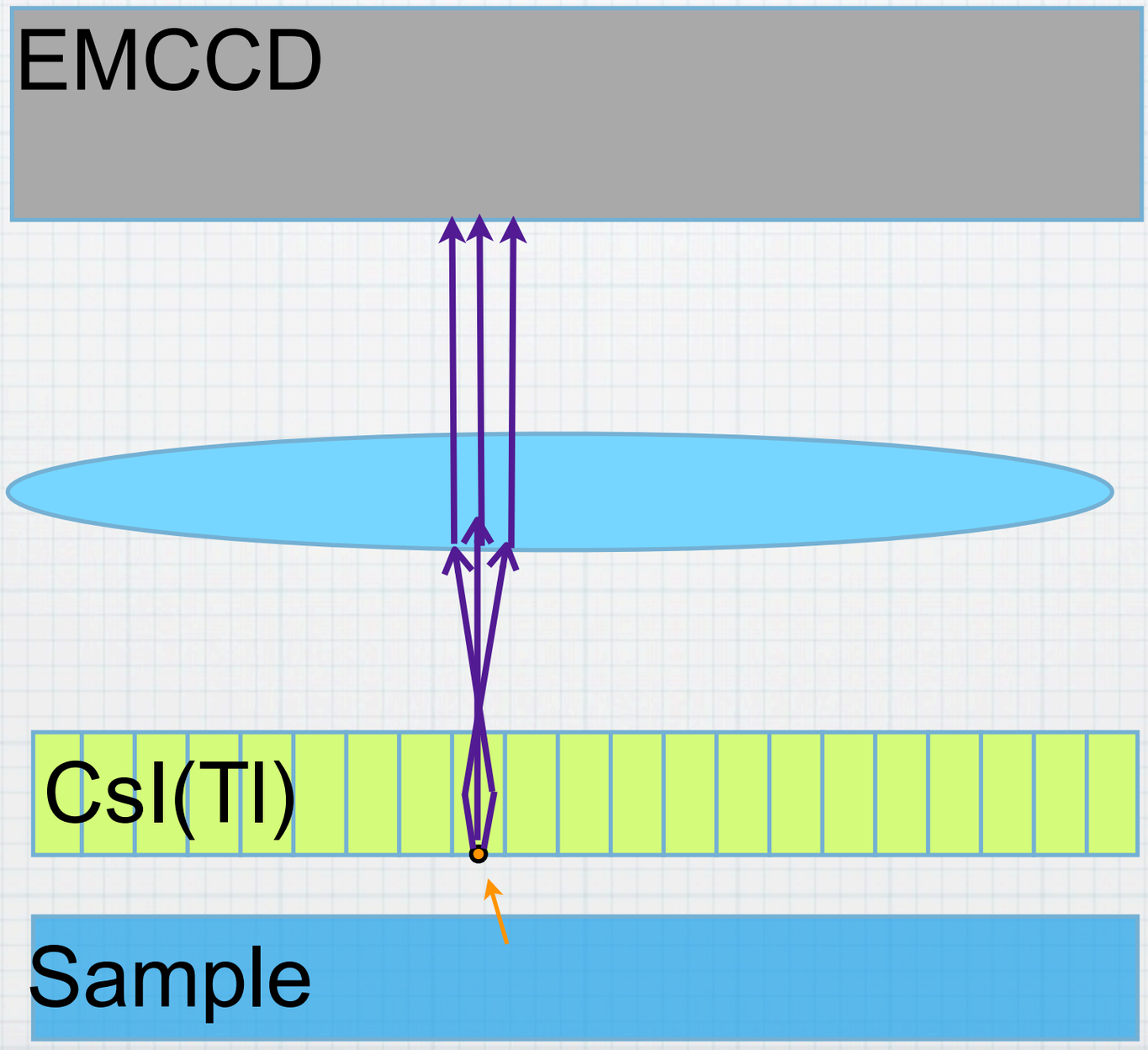


The Signal

EMCCD

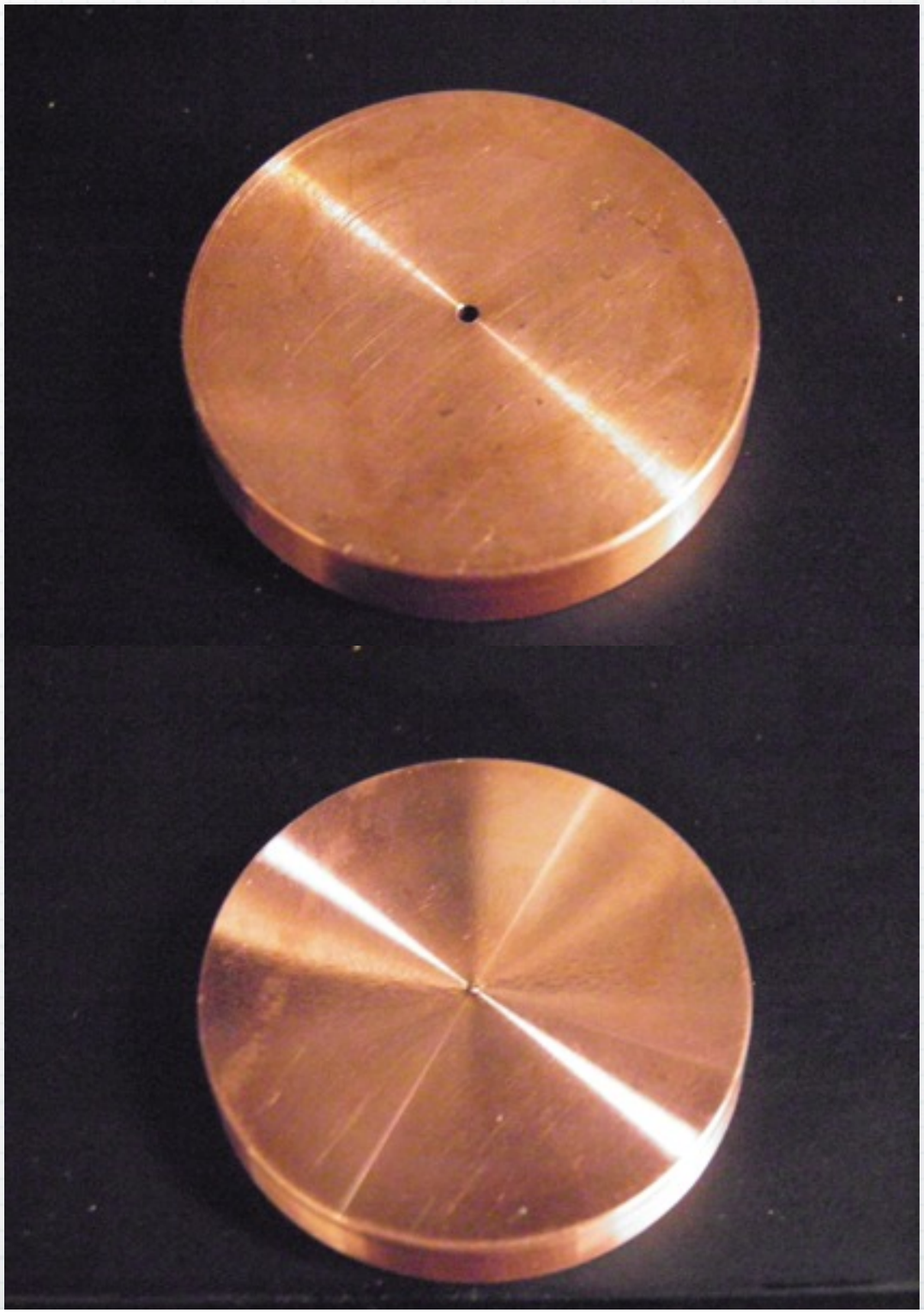


The Signal



Analysis

- Two potential methods:
  - frame-by-frame
  - long-exposure



Analysis - Frame-by-Frame

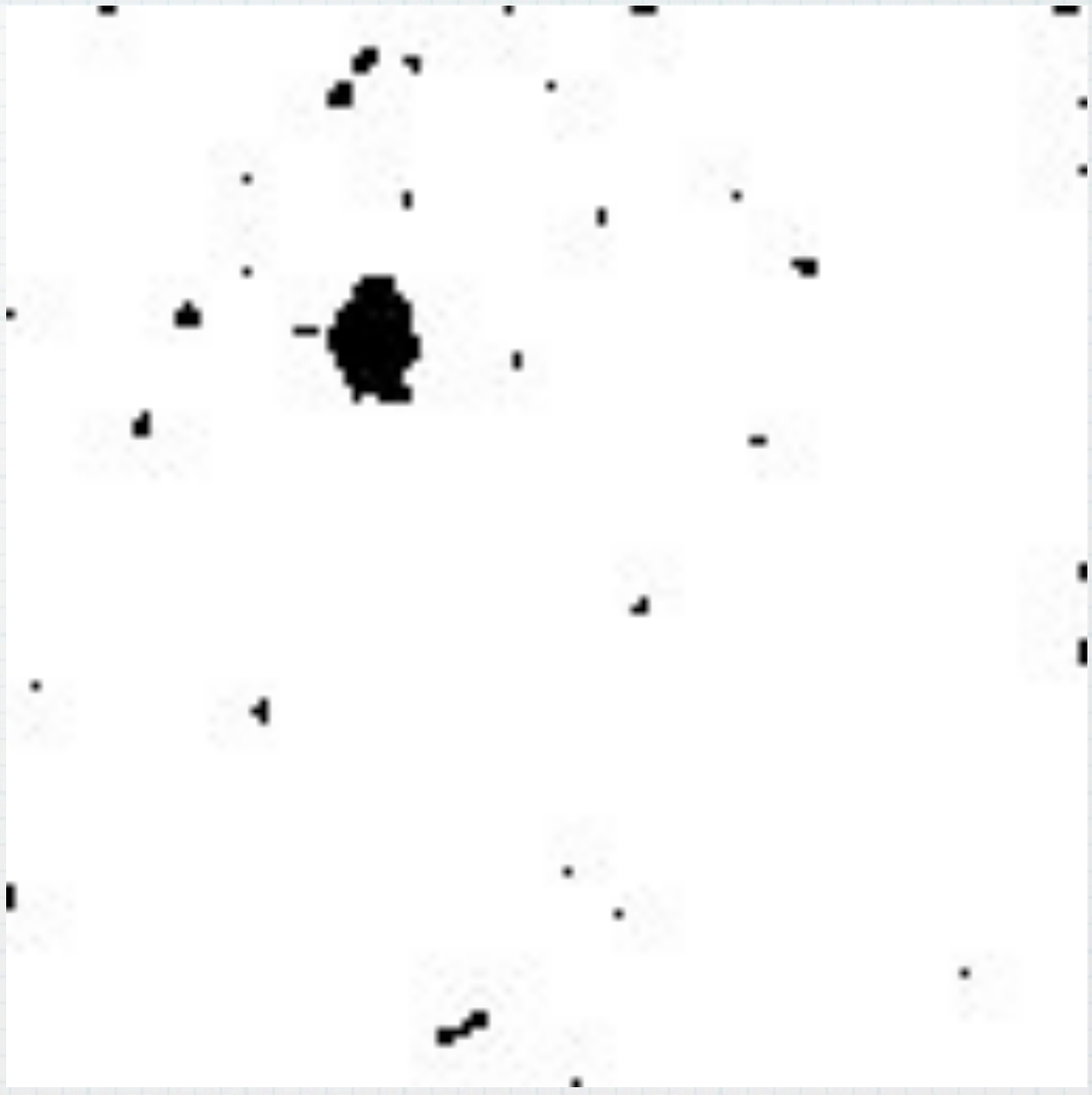
* ^{241}Am source
* 5 MeV alphas make obvious signal

Analysis - Frame-by-Frame

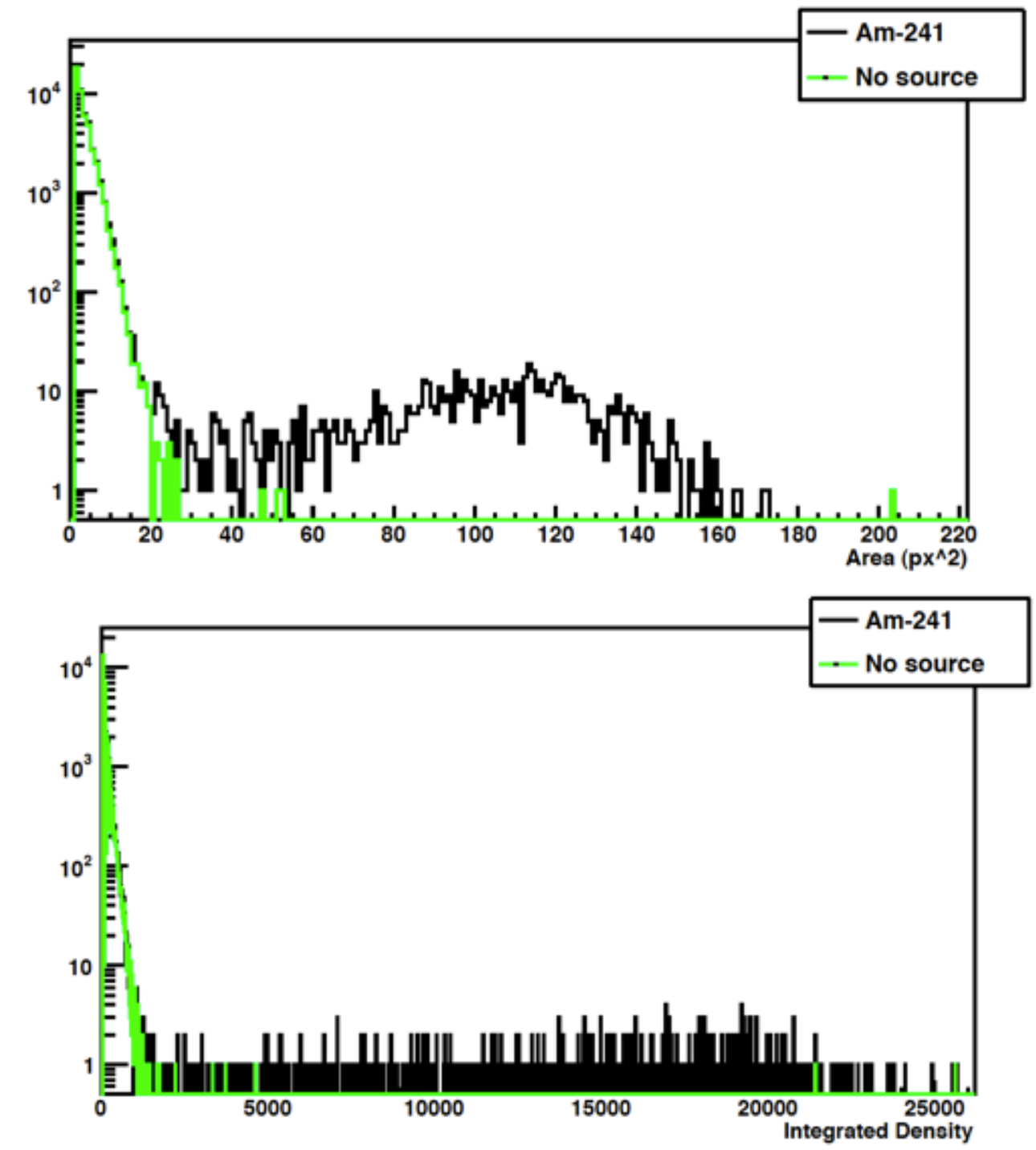
* Subtract background
* Gaussian smear

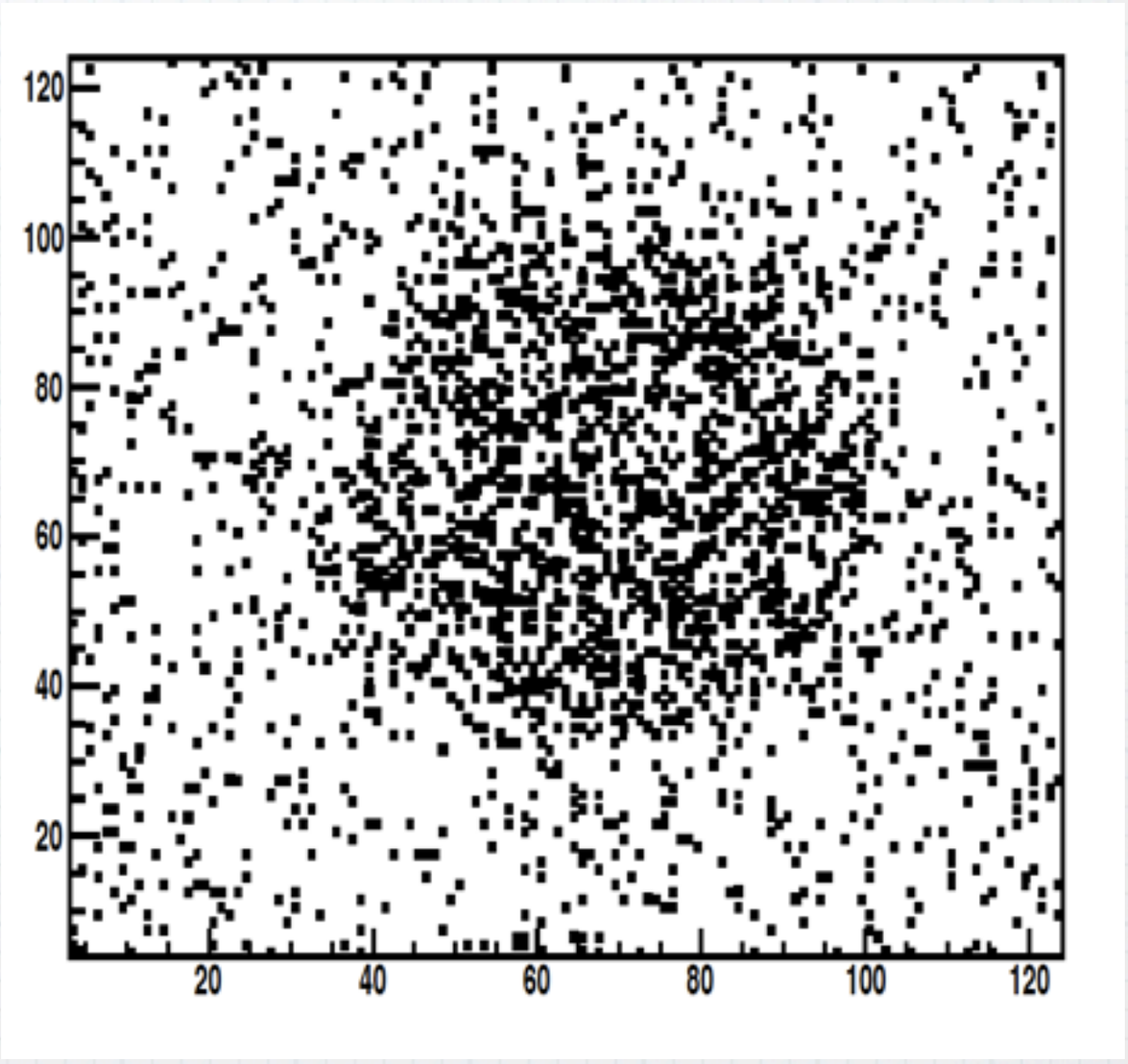
Analysis - Frame-by-Frame

* Apply threshold



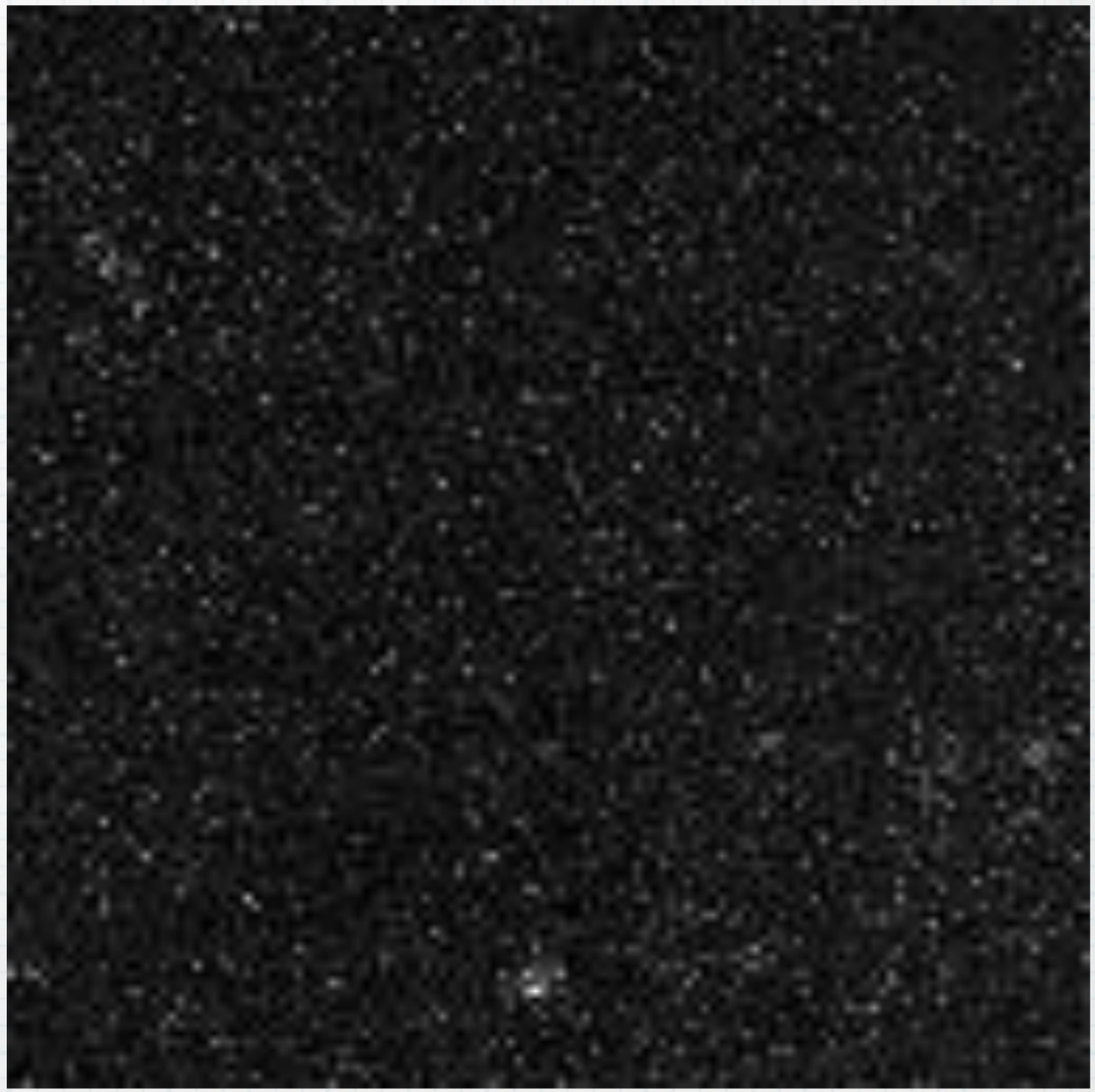
Analysis


Am-241
No source
10^4
10^3
10^2
10
1
0
20
40
60
80
100
120
140
160
180
200
220
Area (px^2)
Am-241
No source
10^4
10^3
10^2
10
1
0
5000
10000
15000
20000
25000
Integrated Density


120
100
80
60
40
20
20
40
60
80
100
120

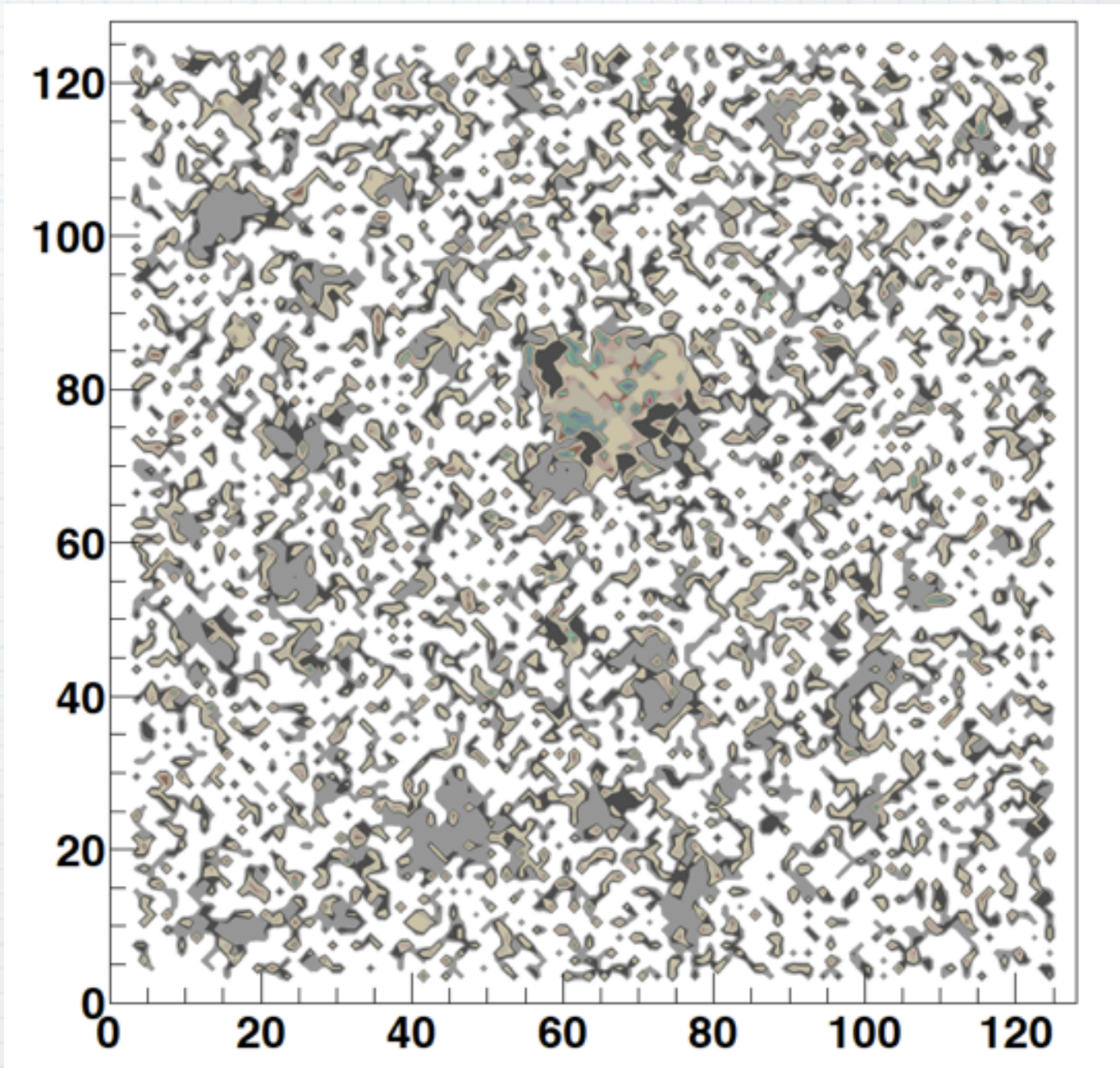
Cs-137

* Electrons emitted in beta decay have endpoint of 513.97 keV (94.70%) with mean energy of 174.32 keV
* Deposit between 80 to 108 keV in 150 μm of CsI
* (Recall ^{14}C has endpoint of 156.48 keV and mean of 49.47 keV)



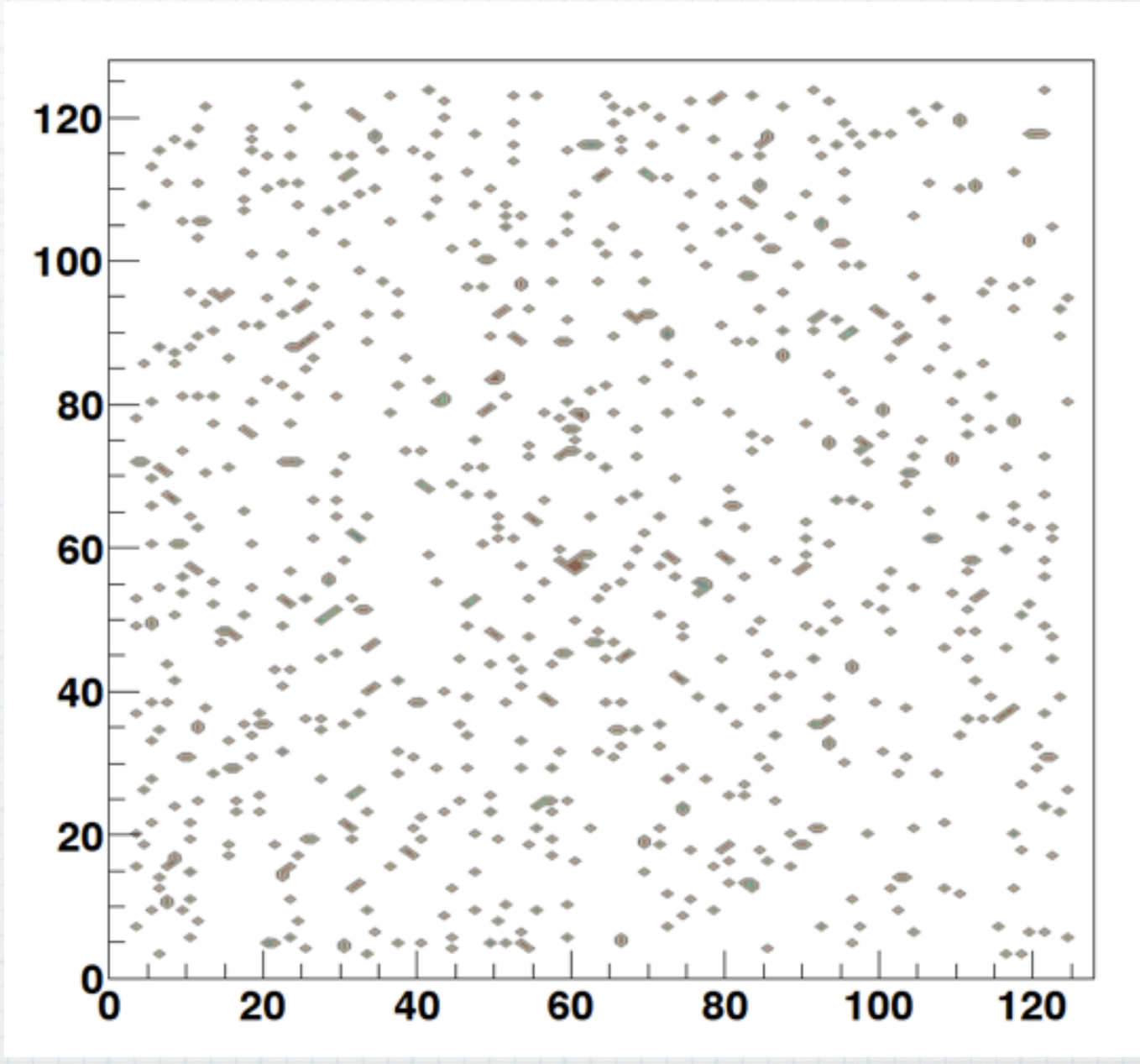
Spectra

- ^{137}Cs with 250 μm mask.

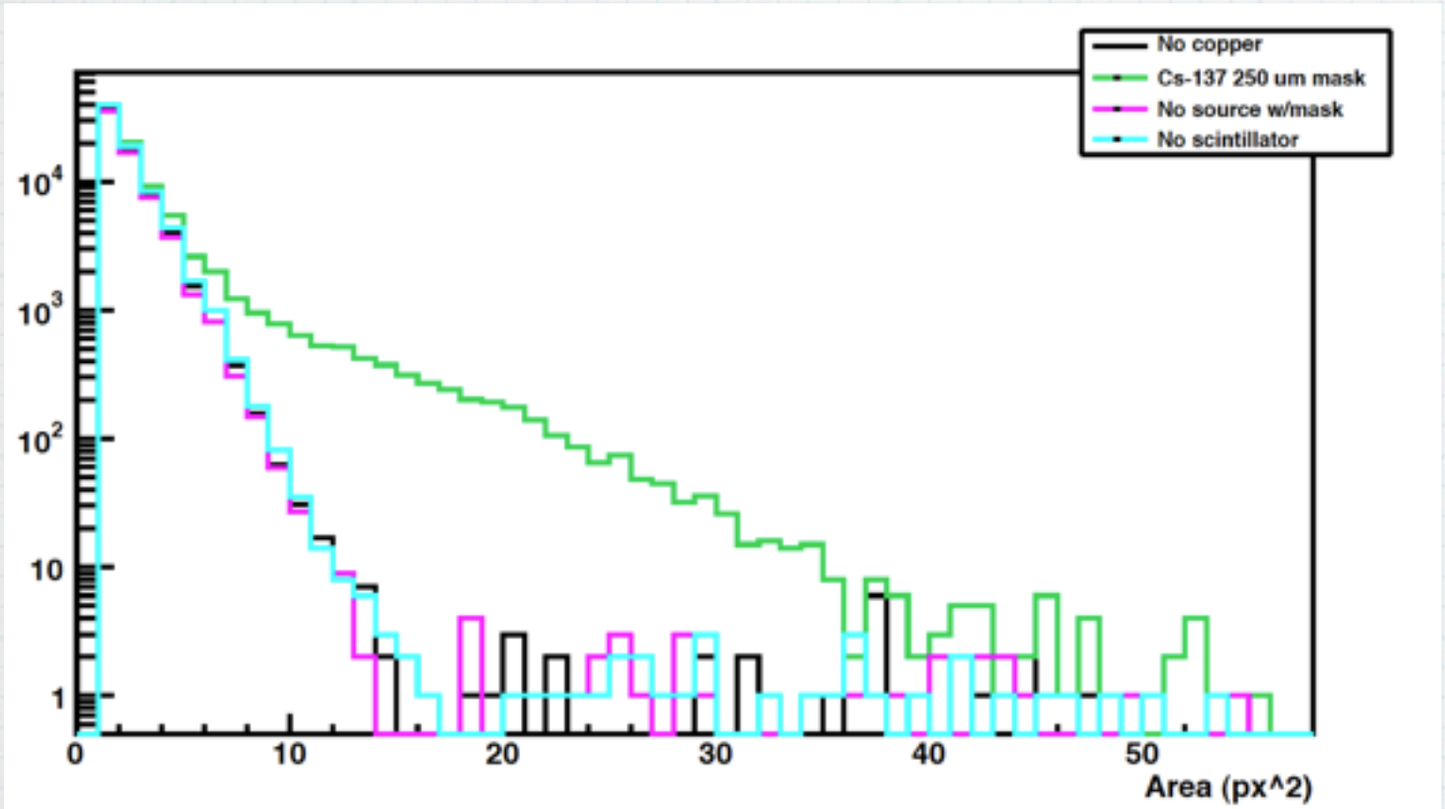


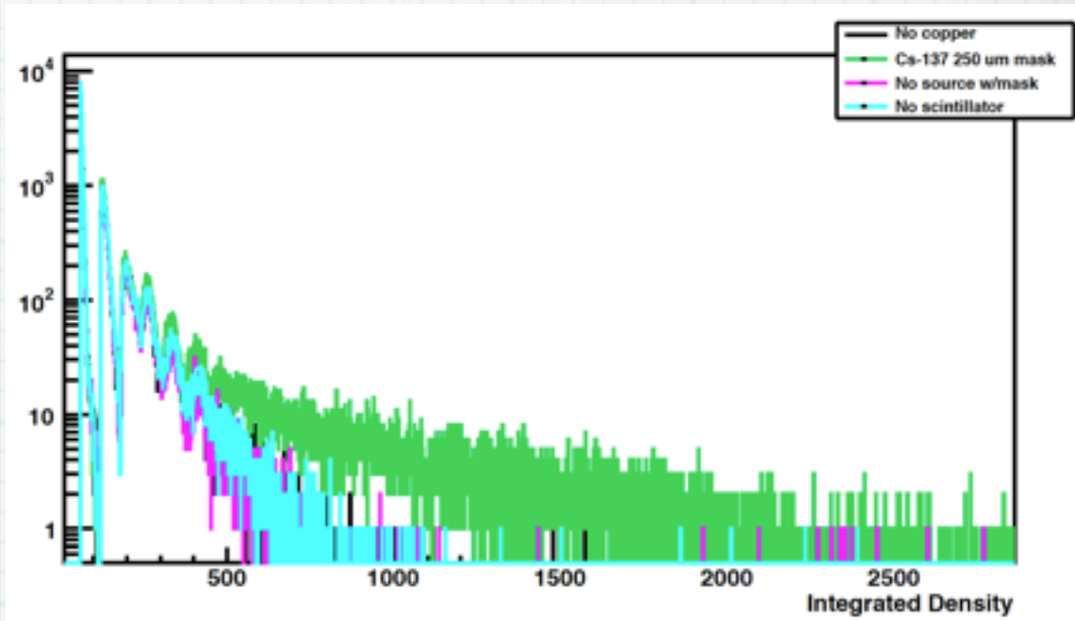
Spectra

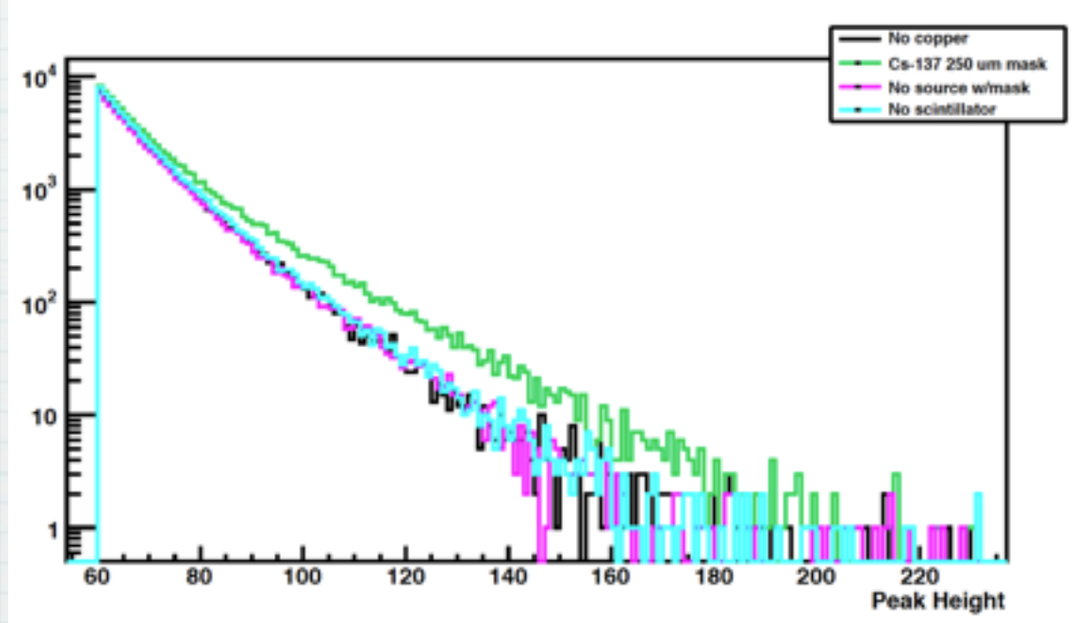
* Background with 250 μm mask.



Spectra

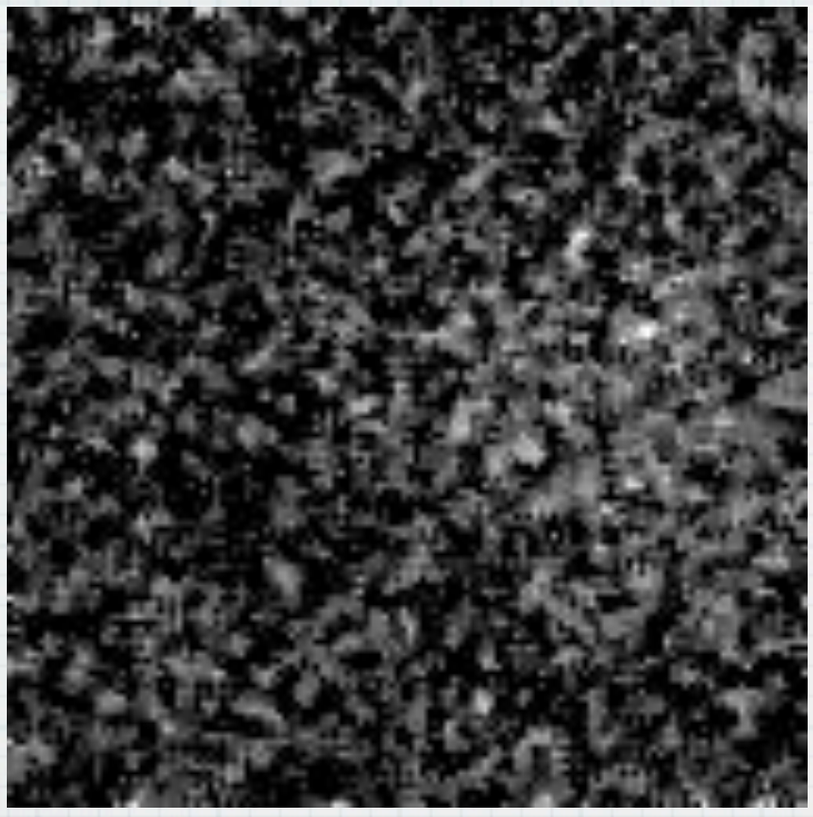

No copper
Cs-137 250 um mask
No source w/mask
No scintillator
10^4
10^3
10^2
10
1
0
10
20
30
40
50
Area (px^2)

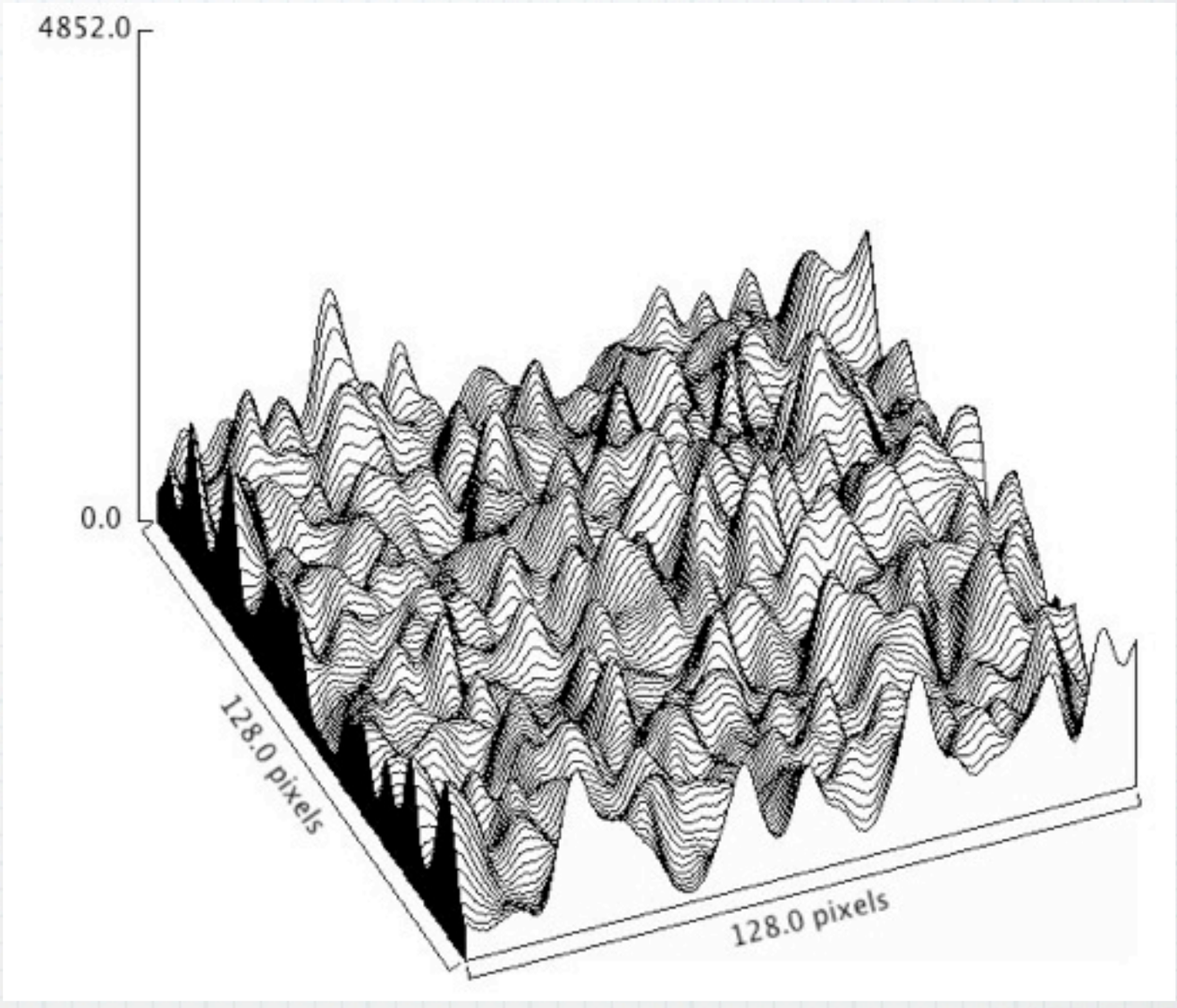

No copper
Cs-137 250 um mask
No source w/mask
No scintillator
10^4
10^3
10^2
10
1
500
1000
1500
2000
2500
Integrated Density


No copper
Cs-137 250 um mask
No source w/mask
No scintillator
10^4
10^3
10^2
10
1
60
80
100
120
140
160
180
200
220
Peak Height

Long Exposures

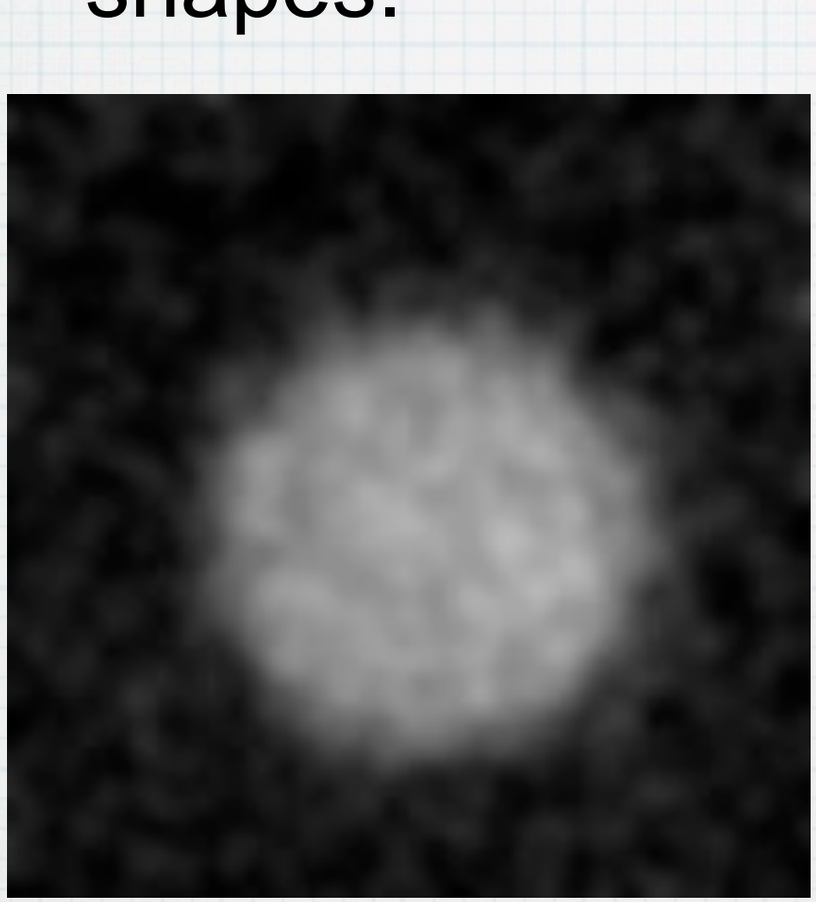
* Can also take long exposures to image shapes.

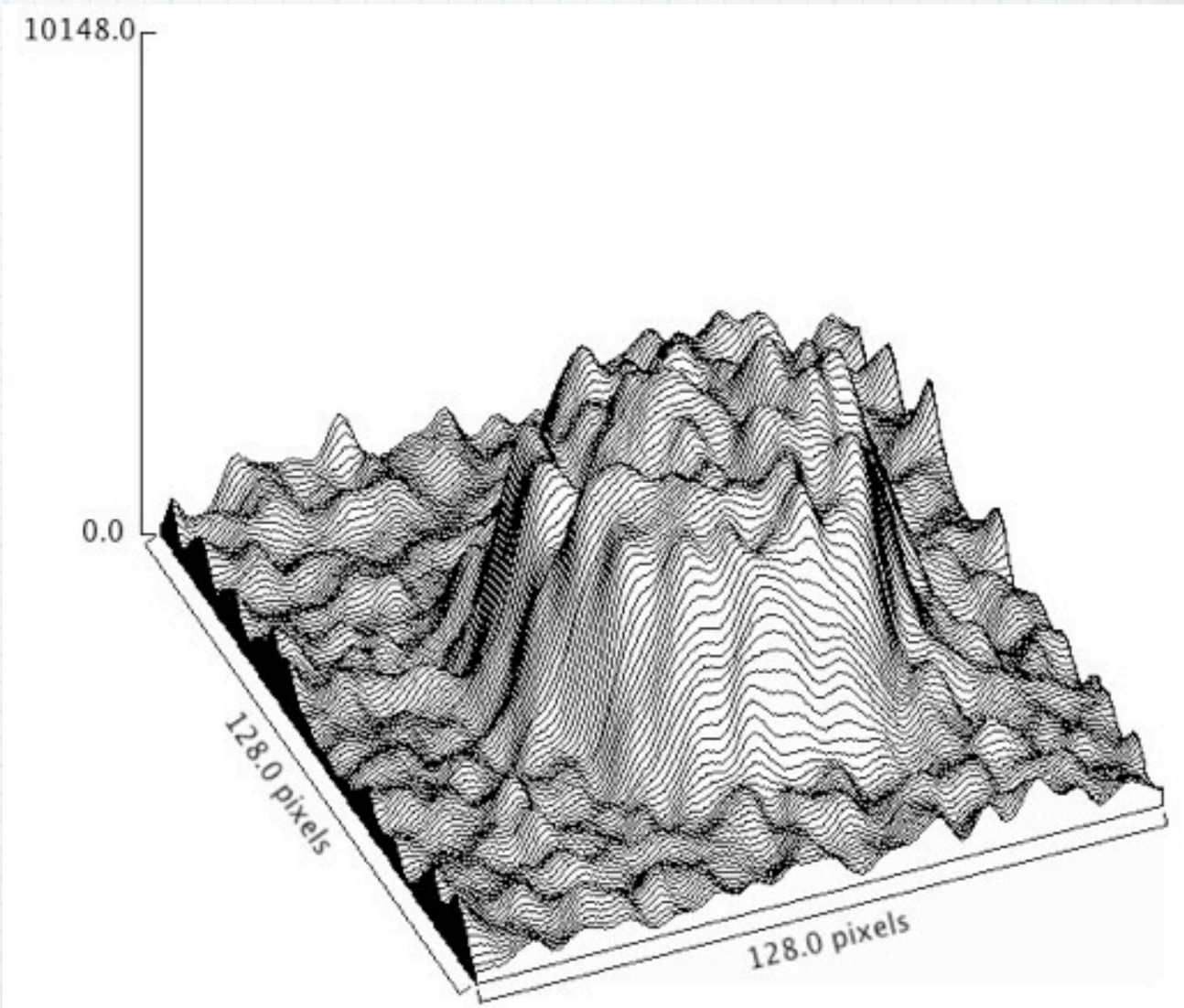




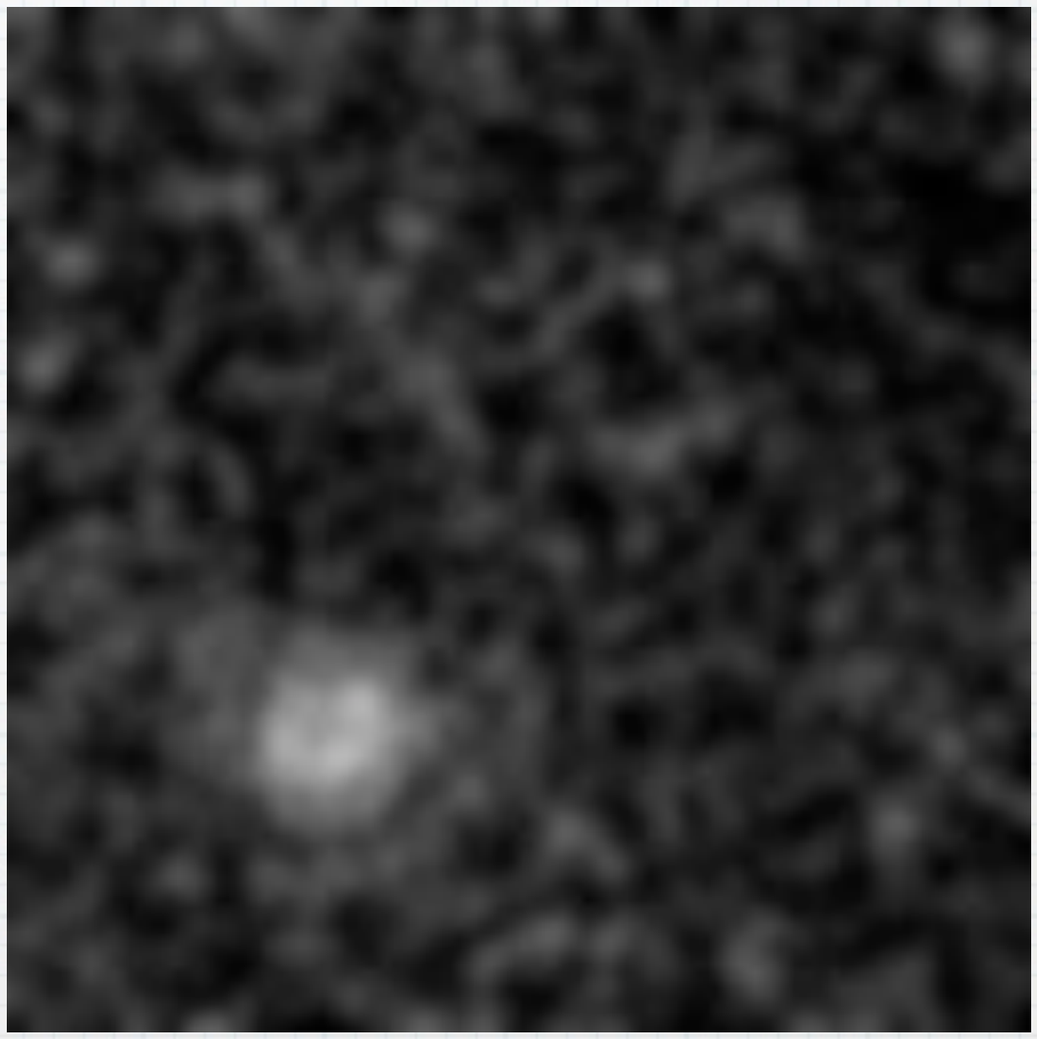
Long Exposures

* Can also take long exposures to image shapes.





Long Exposures

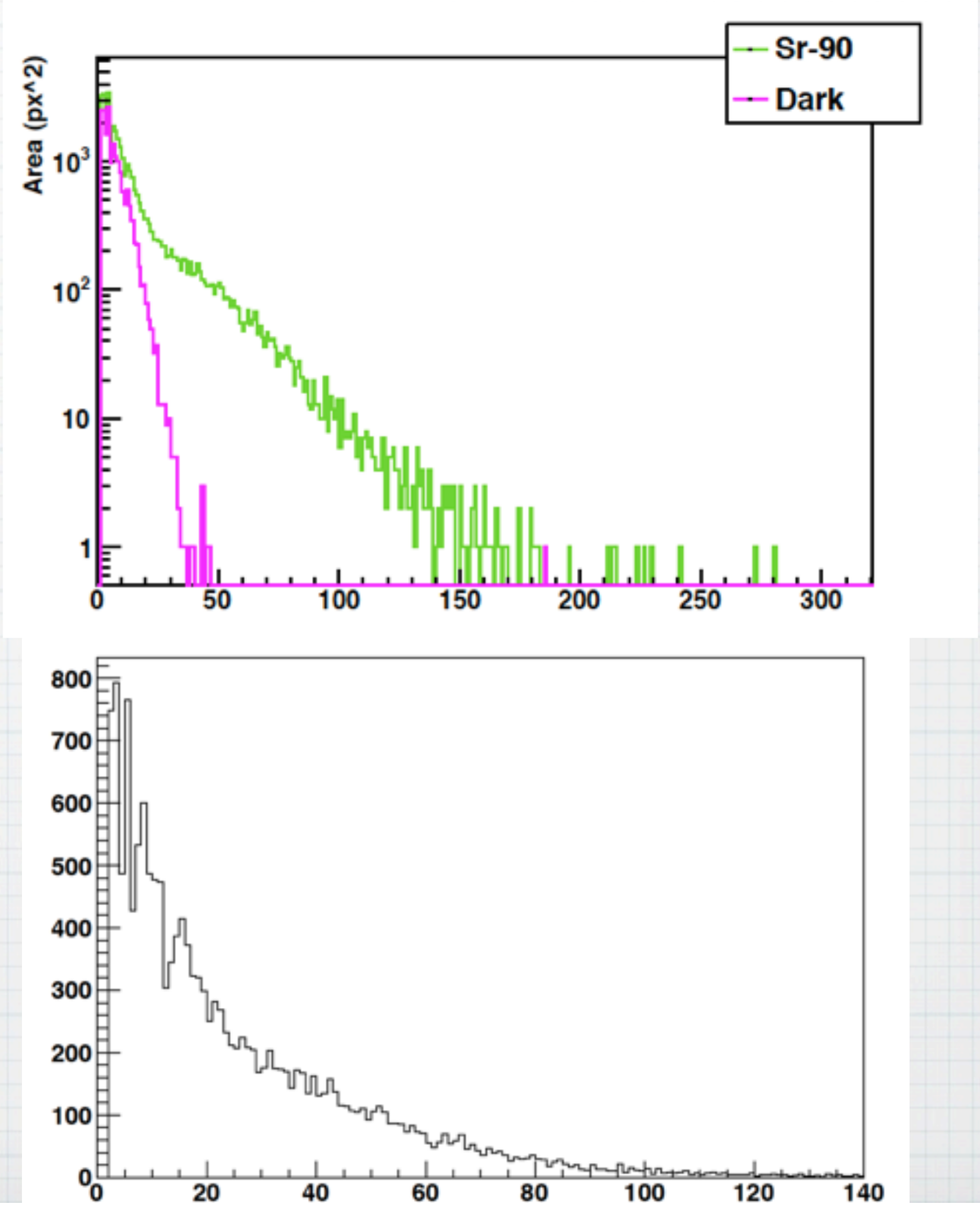


* 250 μm mask with 3 minute exposure.

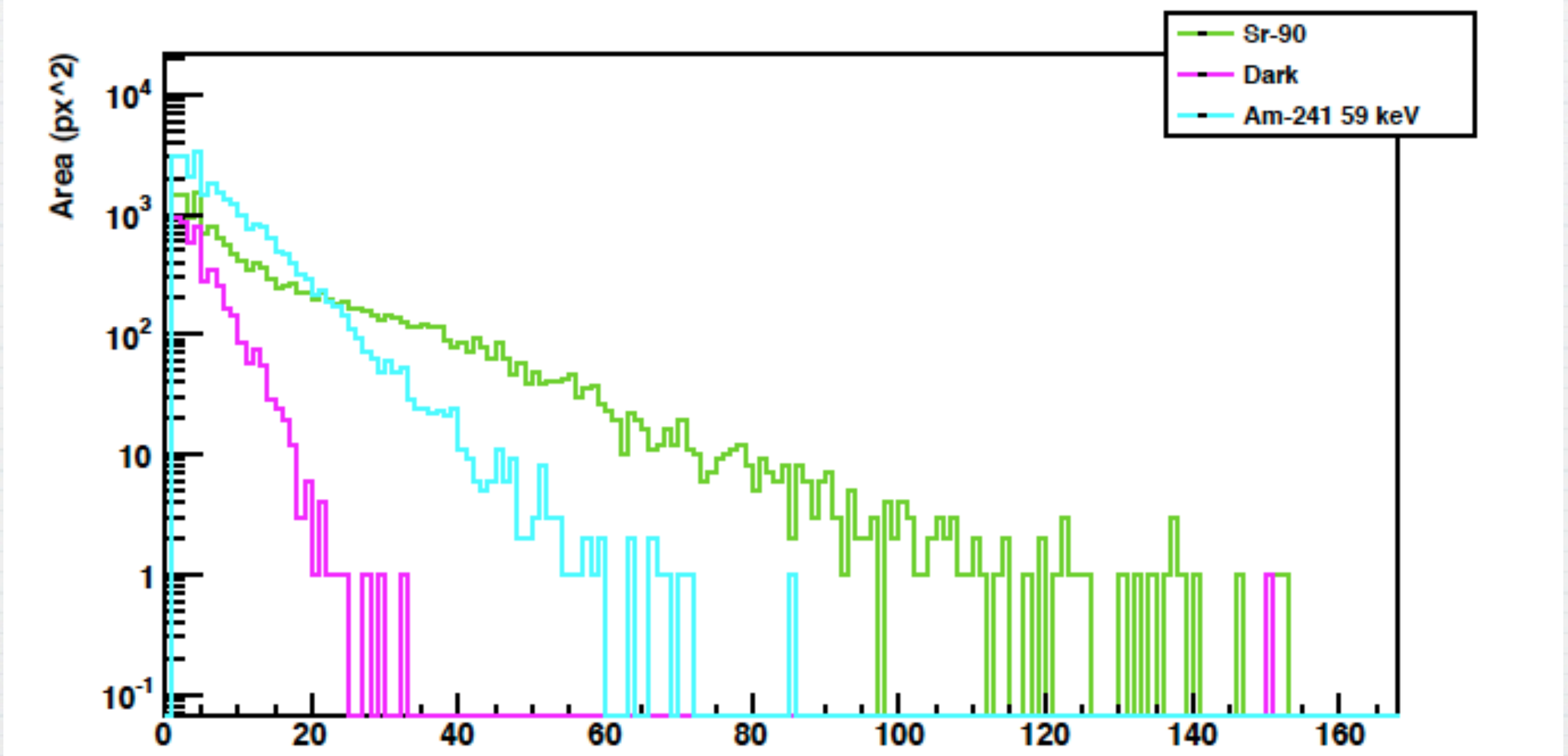
Sr-90

* emits a 195.8 keV (mean), 546.0 keV (endpoint) beta to ^{90}Y.

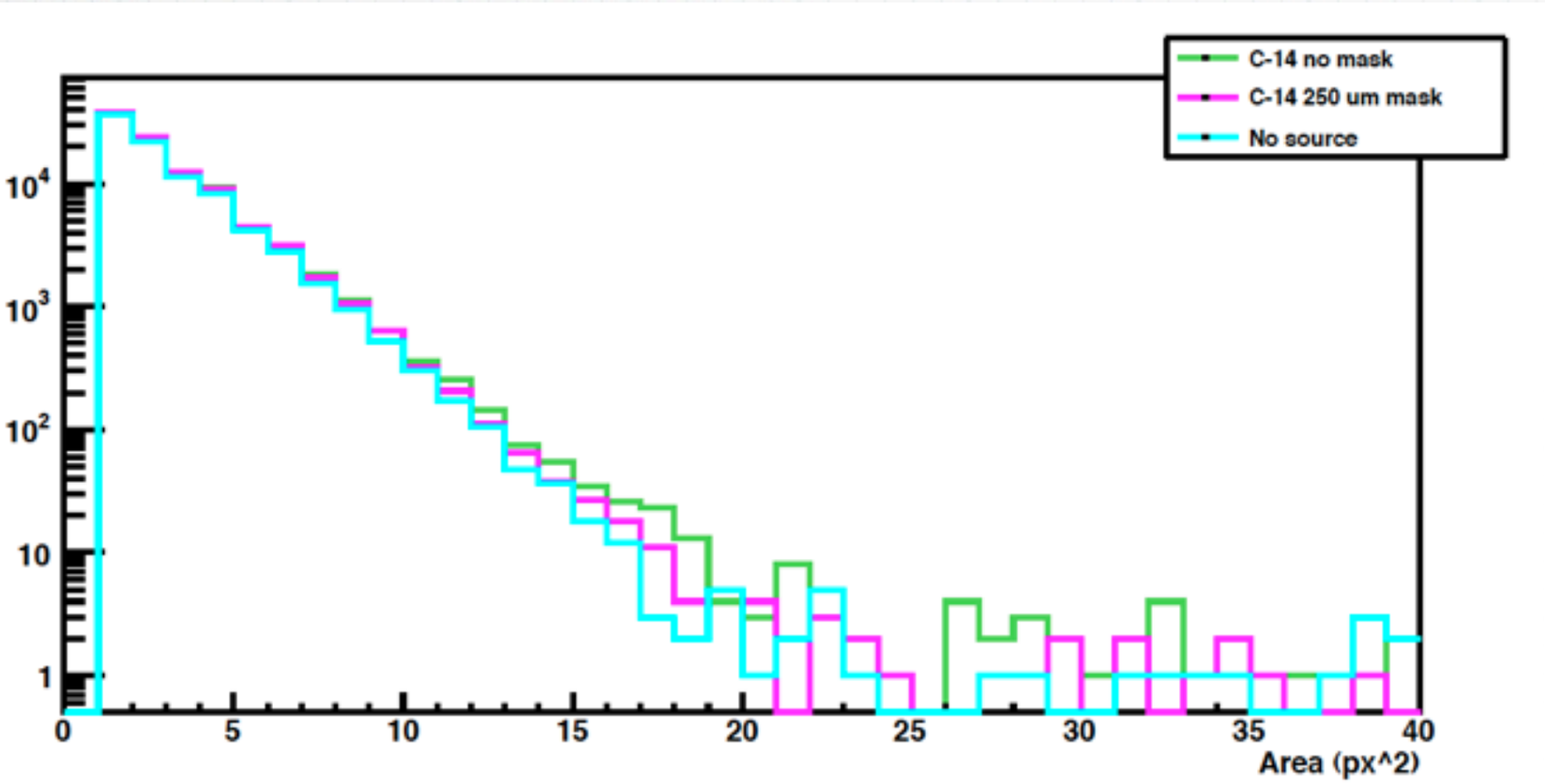
* 195.8 keV electron should deposit about 109.4 keV in 150 um of CsI.



59 keV gamma from ^{241}Am

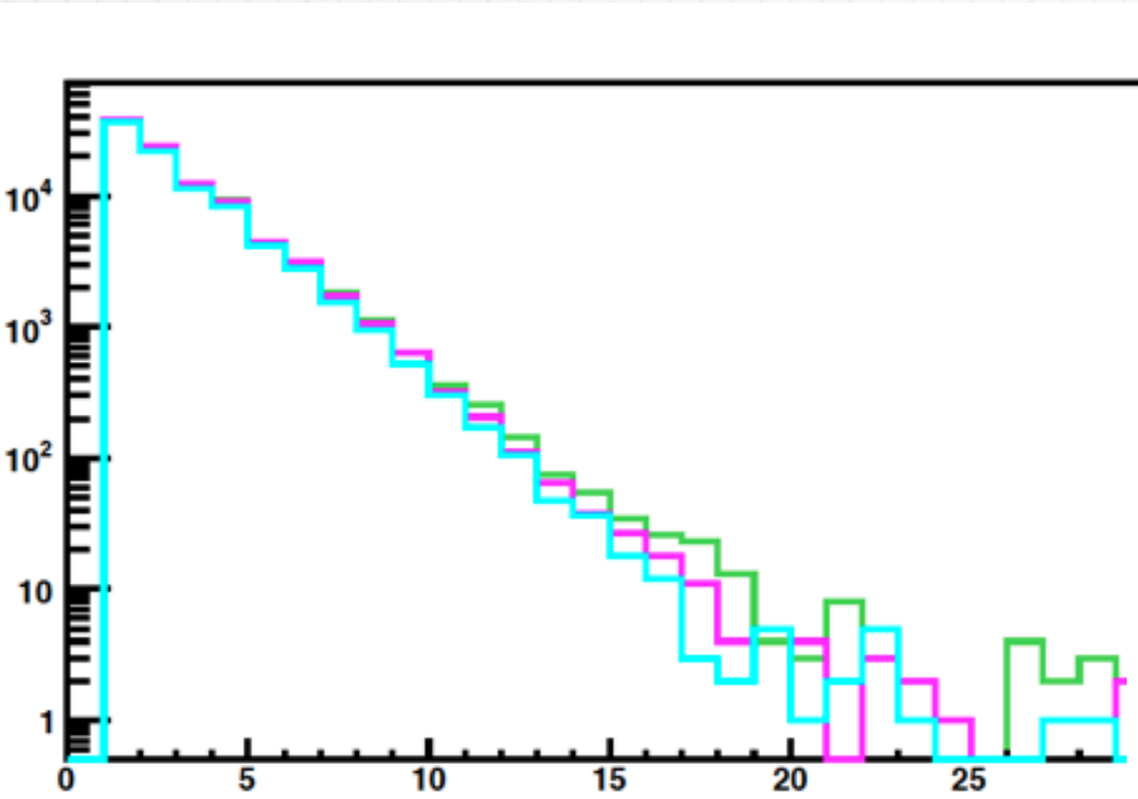


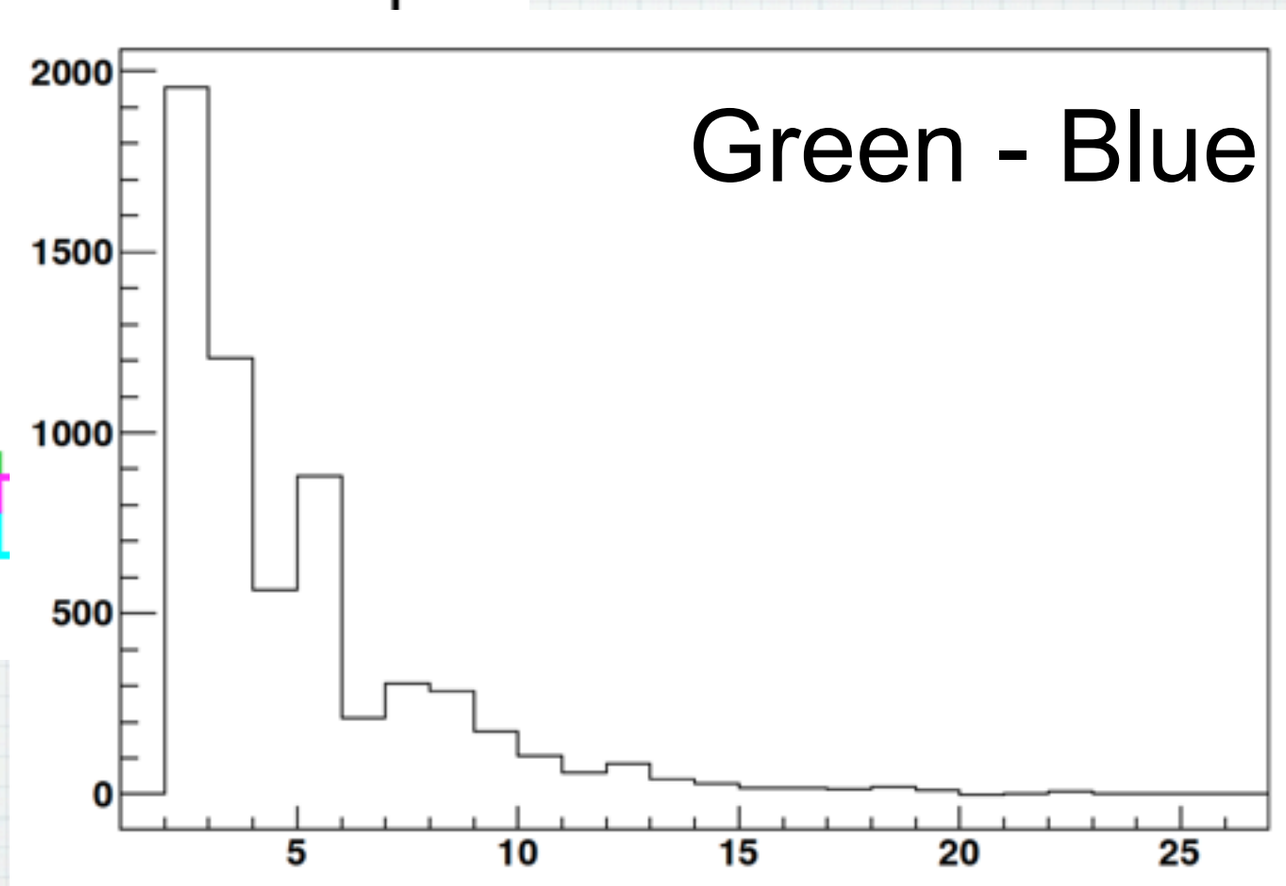
C-14?



45 nCi source

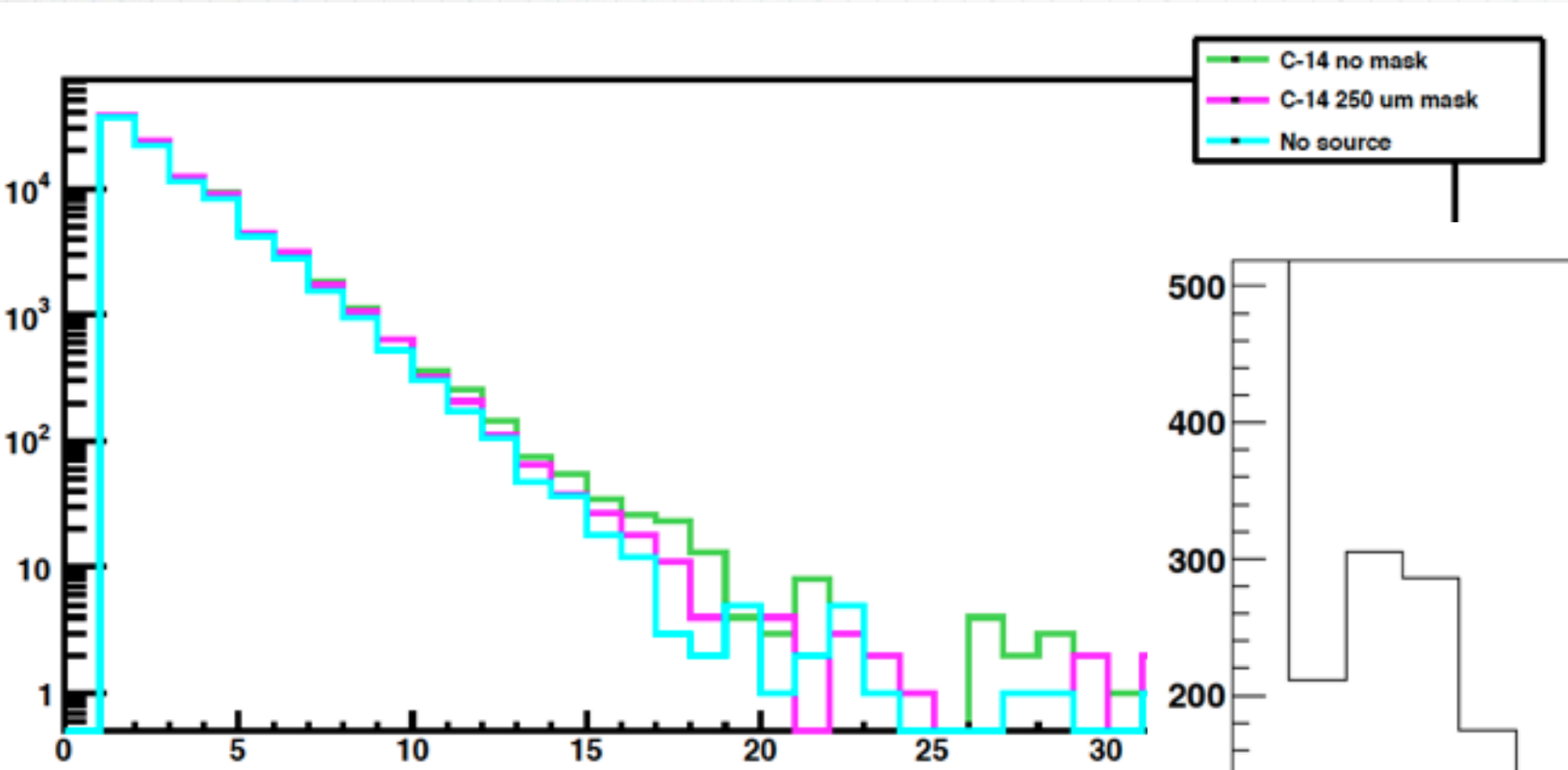
C-14?

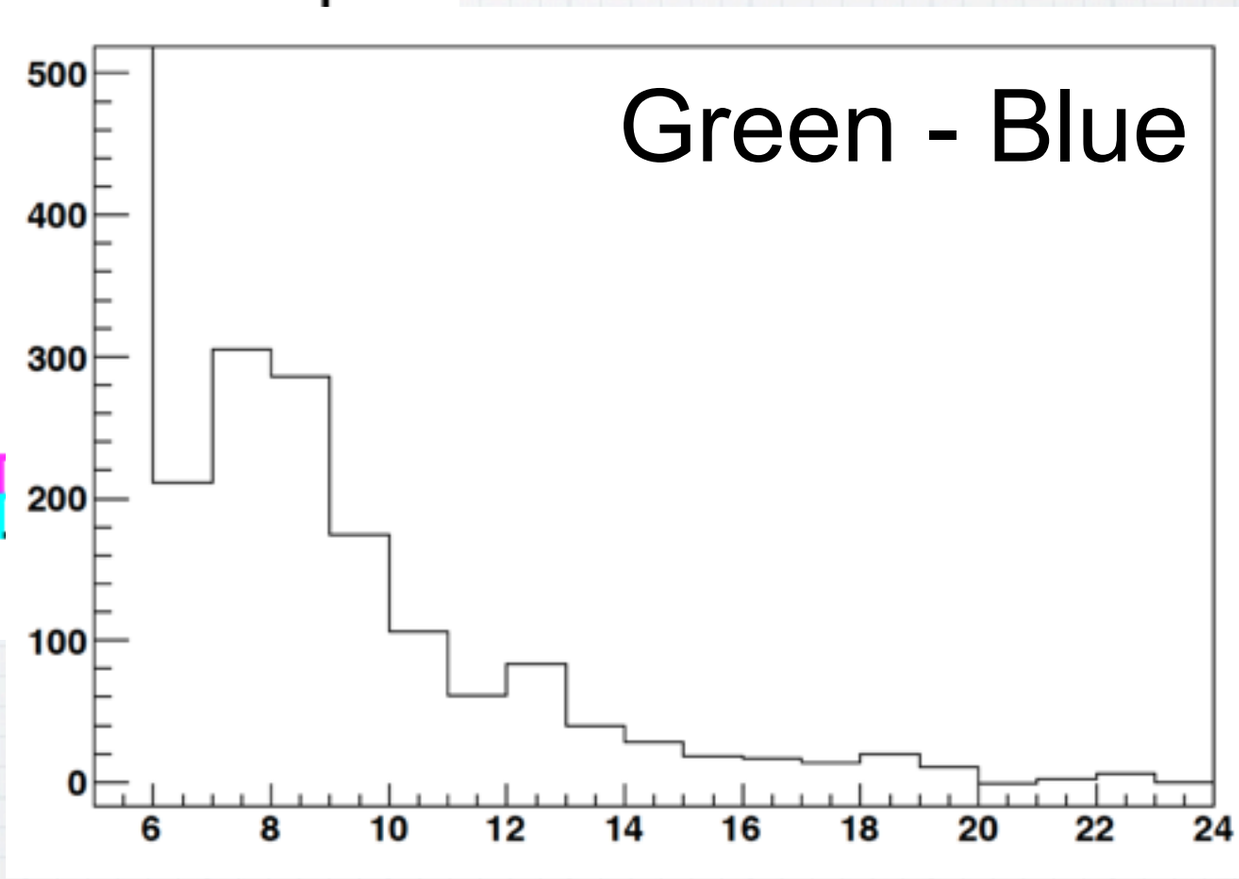




Green - Blue

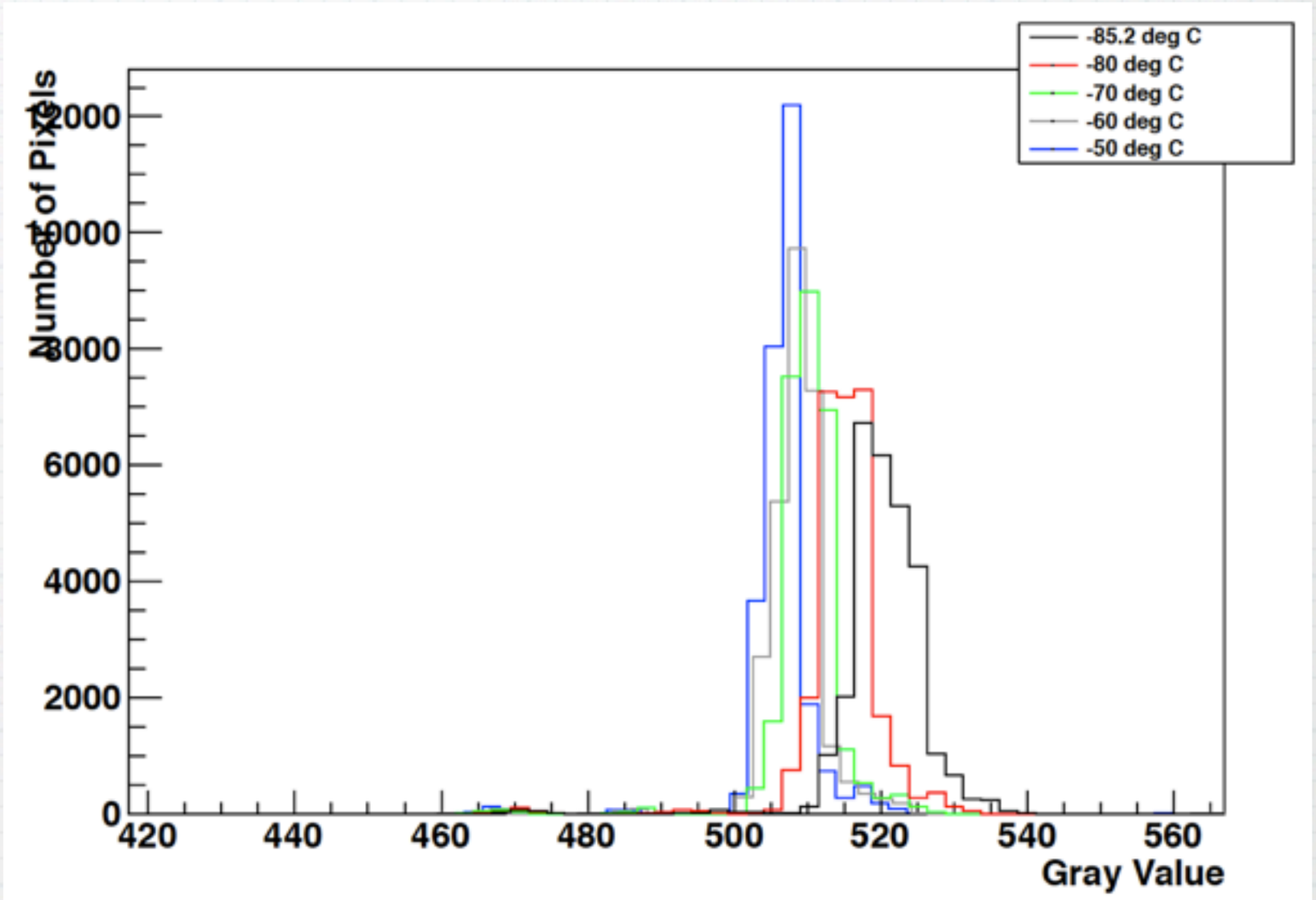
C-14?


C-14 no mask
C-14 250 um mask
No source
10^4
10^3
10^2
10
1
0
5
10
15
20
25
30


Green - Blue
500
400
300
200
100
0
6
8
10
12
14
16
18
20
22
24

The background problem

- As seen, it's very difficult to pull out the ^{14}C signal.
- We have asked around the lab if anyone has a stronger ^{14}C source for testing.
- Meanwhile, we continue to investigate the noise in the camera

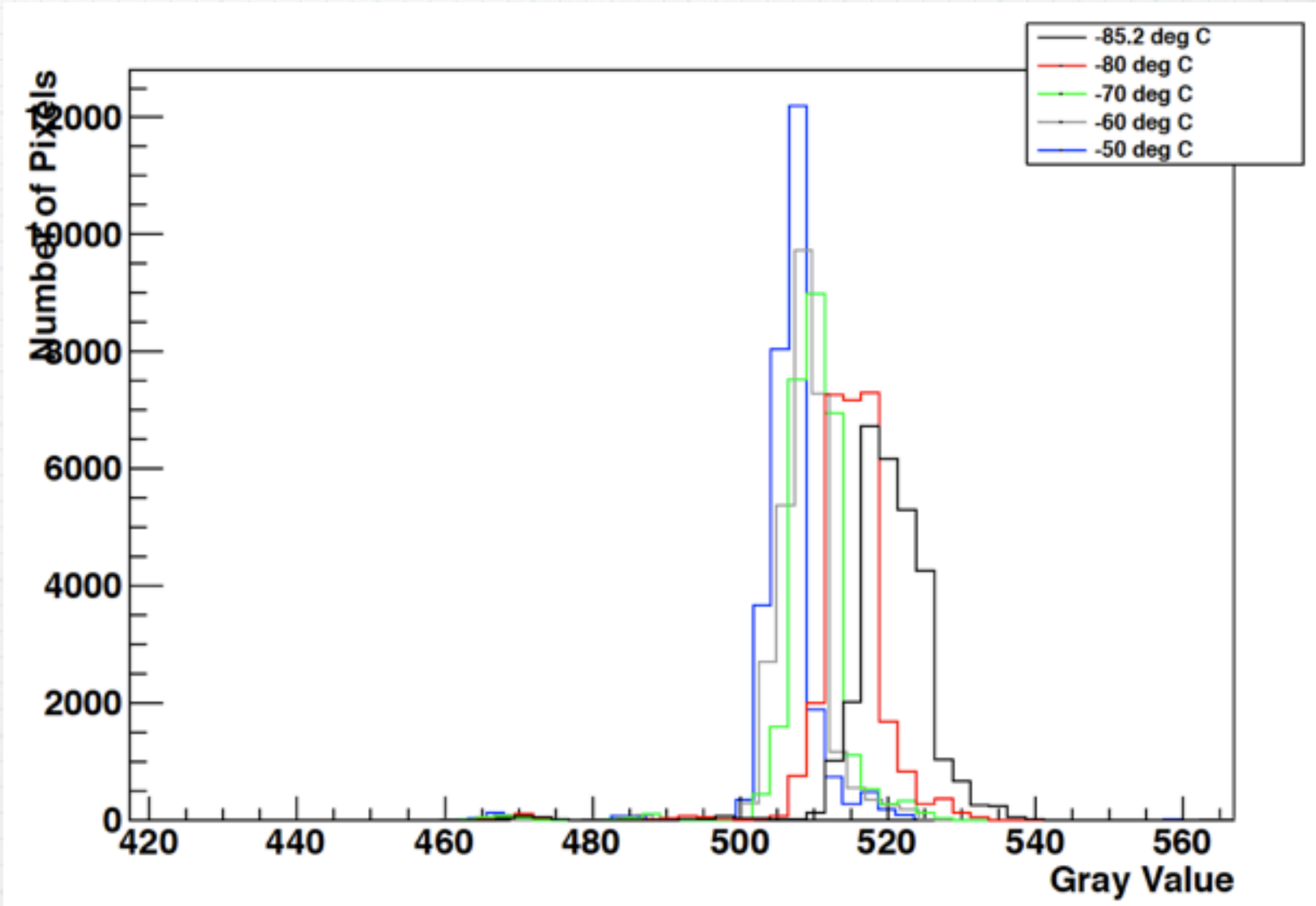


The background problem

* Naively, one would expect background to be lower at lower temperatures

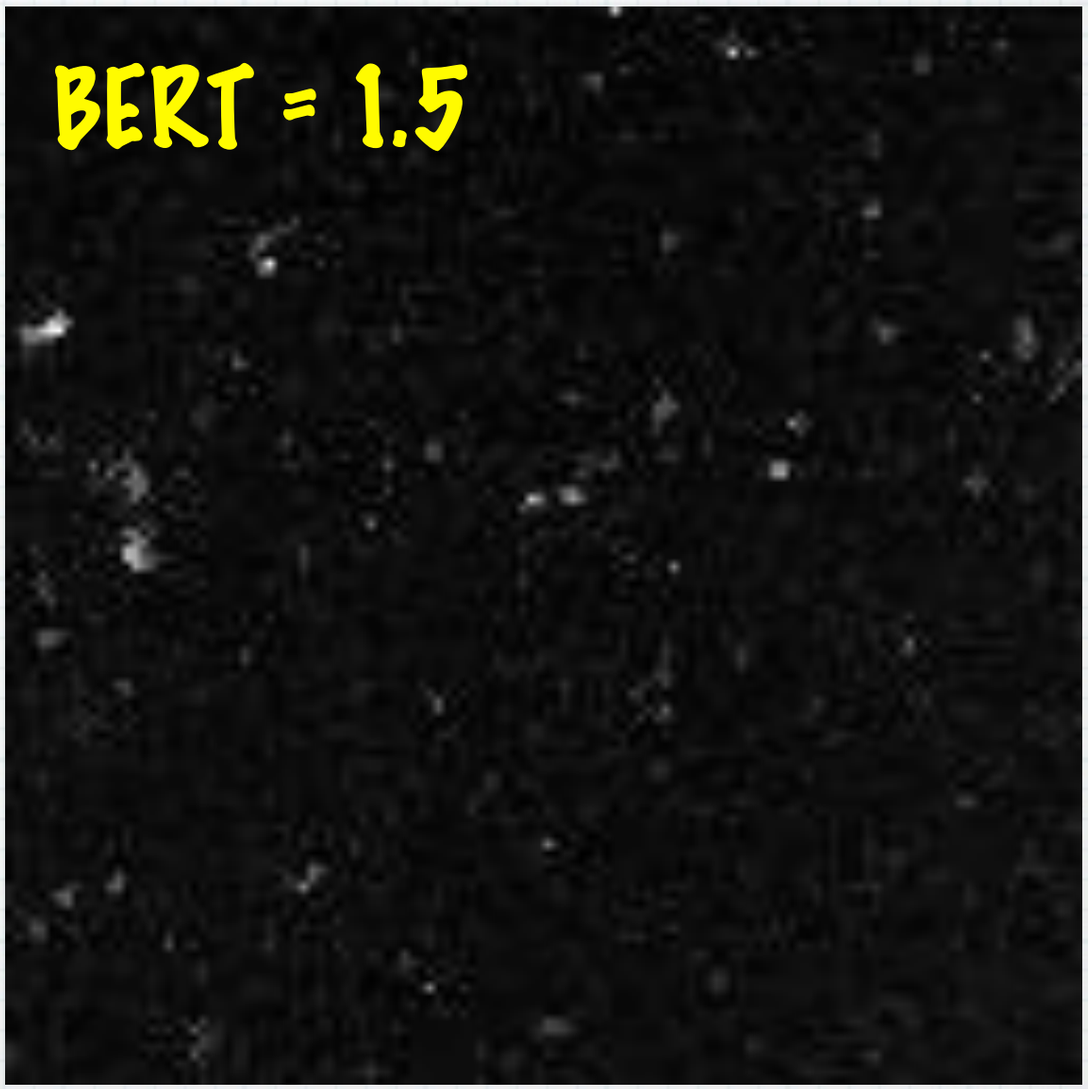
* This is generally true, however EMCCDs have a different feature - clock-induced charge (CIC)

* At low temperatures and high gain, this is a noticeable effect.

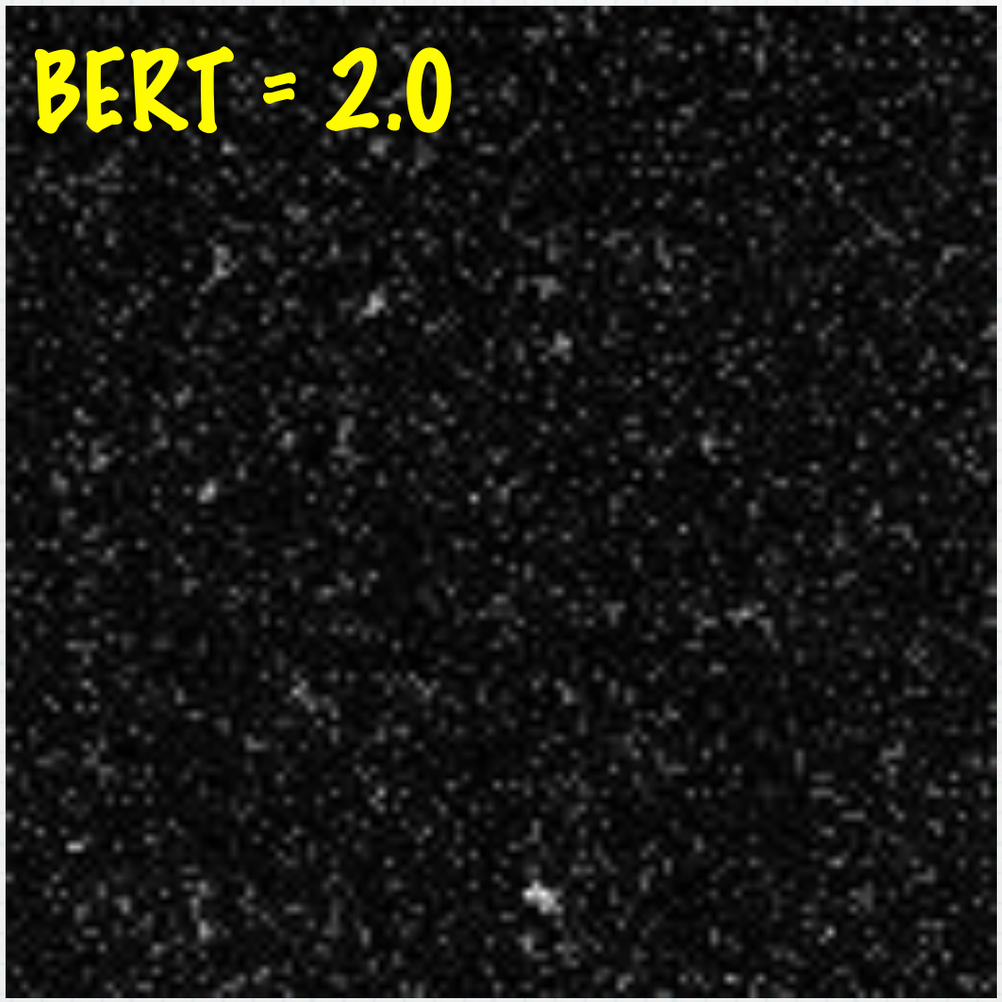


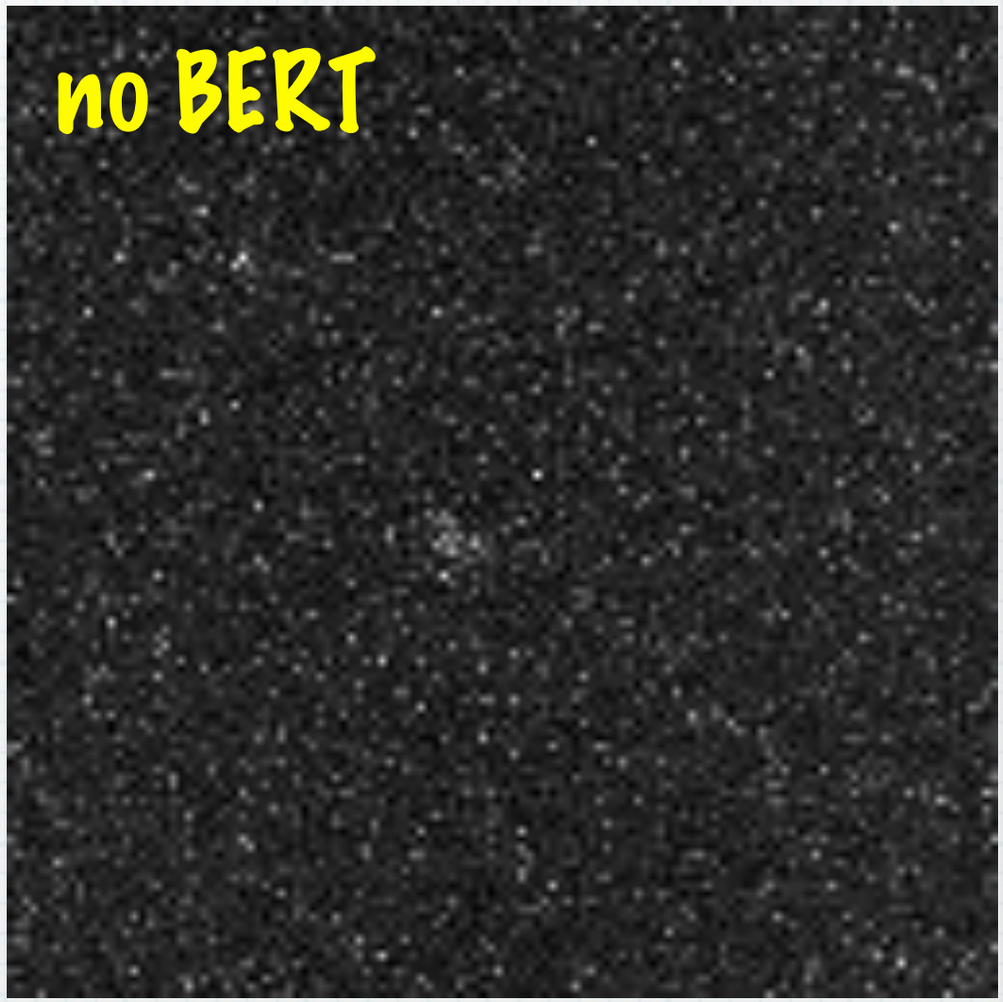
BERT

- One way to mitigate CIC is to utilize a tool in the microscope software - Background Event Reduction Techology (BERT)
- BERT sets up a median filter to remove spurious events in real time
  - each pixel is compared to surrounding pixels
  - if the pixel is greater than the median value of itself and surrounding pixels by a user-defined threshold, it is replaced by the median value



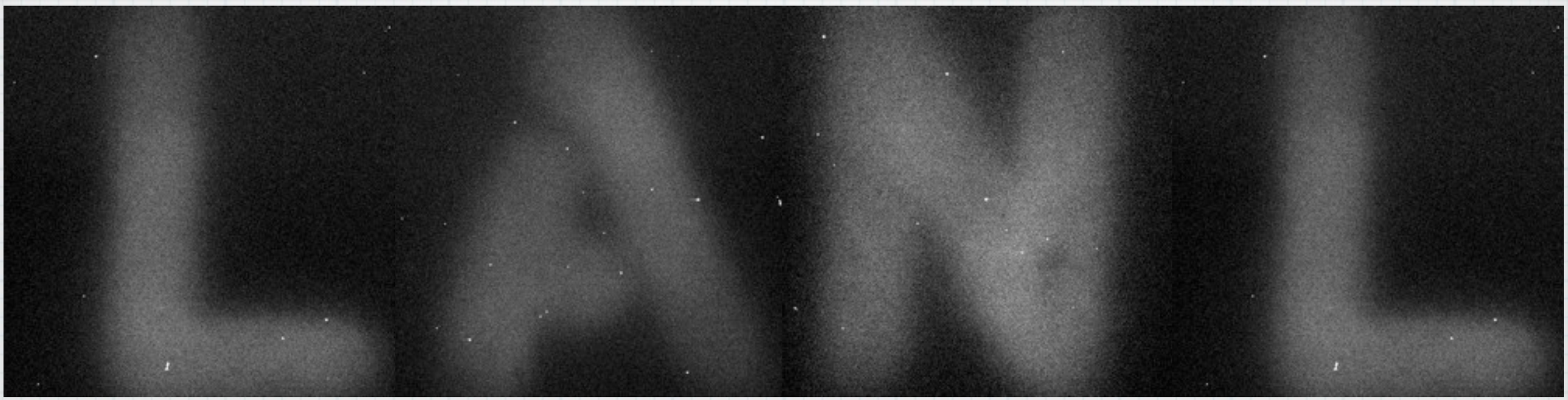
BERT side-by-side





To Do

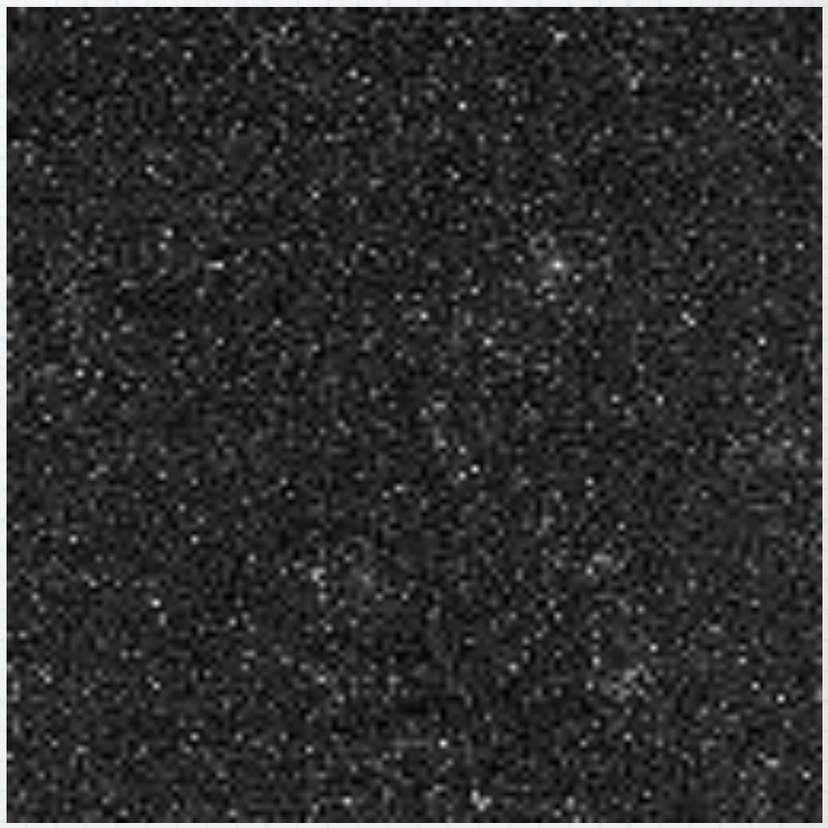
* Detect ^{14}C convincingly
* Continue to test resolution
* Continue to improve frame-by-frame analysis
* Study sample slide - should arrive this week!



Thank you!

^{241}Am
(4.5 nCi)

Background



^{137}Cs
(5 µCi)

Supported by DOE-BER