



## Reference (2)

Jeff (CHI-HSUAN HO)



# **Intensity Extraction and Data collecting Software – Summit Grid Review, Improve & Evaluate**

Jeff (CHI-HSUAN HO)

# Gridding Development



- **Summit.Grid**

### o Chip Spec Parameters Setup

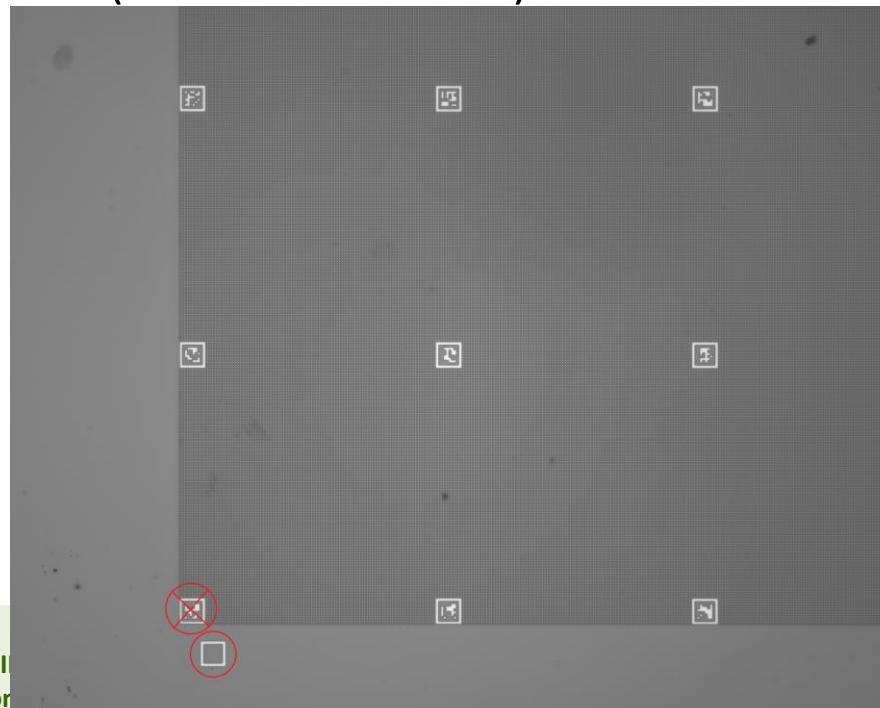
```
"name": "banff",
"w": 2480,
"h": 2480,
"w_cl": 496,
"h_cl": 496,
"cell_w_um": 4,
"cell_h_um": 4,
"space_um": 1,
"location_marker": {
    "template": "resource/banff/pat_white.tif",
    "mask": "resource/banff/pat_white_mask.tif",
    "w": 60,
    "h": 60
},
"shooting_marker": {
    "origin_desc": "center of the chip",
    "type": "regular_matrix",
    "mk_pats": [
        {
            "filter": 0,
            "w_um": 60,
            "h_um": 60,
            "path": "resource/banff/pat_white.tif",
            "mask": "resource/banff/pat_white_mask.tif"
        },
        {
            "um2px_r": 2.68,
            "path": "resource/banff/pat_2_68.tif"
        },
        {
            "um2px_r": 2.41,
            "path": "resource/banff/pat_2_41.tif"
        }
    ],
    "chip": "banff33",
    "view": {
        "offset": [ 0, 0 ],
        "layout": [ 1, 1 ],
        "stride": [ 810, 810 ]
    }
},
"scan_channel": {
    "name": "White8",
    "pixel_format": "Mono8",
    "gain": 0,
    "exposure_time_abs": 500,
    "camera_delay_time": 1,
    "filter": 0
},
"module_parameters": {
    "sweep_distance": 320,
    "sweep_stepsize": 20,
    "boxgrid": {
        "layout": [ 3, 3 ],
        "stride": [ 405, 405 ],
        "cells": [ 120, 120 ],
        "enable": [ 0, 2, 6, 8 ]
    }
}
```

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- Summit.Grid
  - Bugfix for wrong nms\_count (WARNING for S1C)
    - Original
  - Noise influence (WARNING for Y2B)

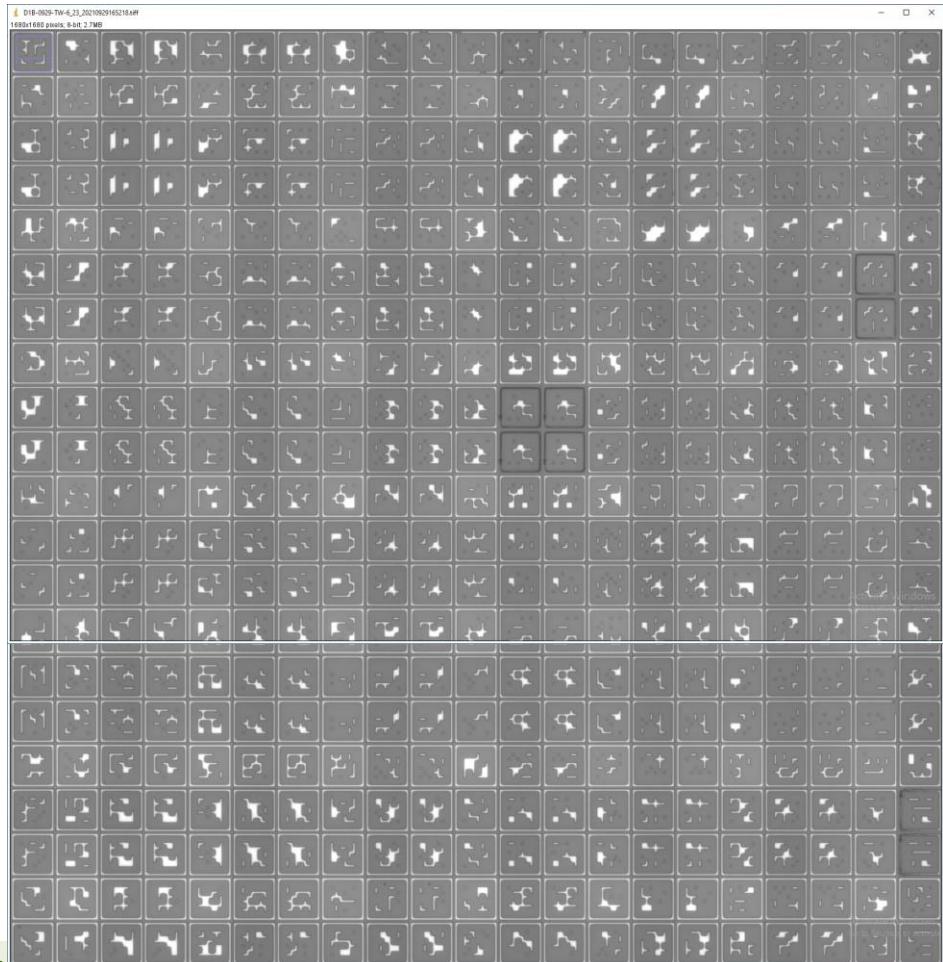
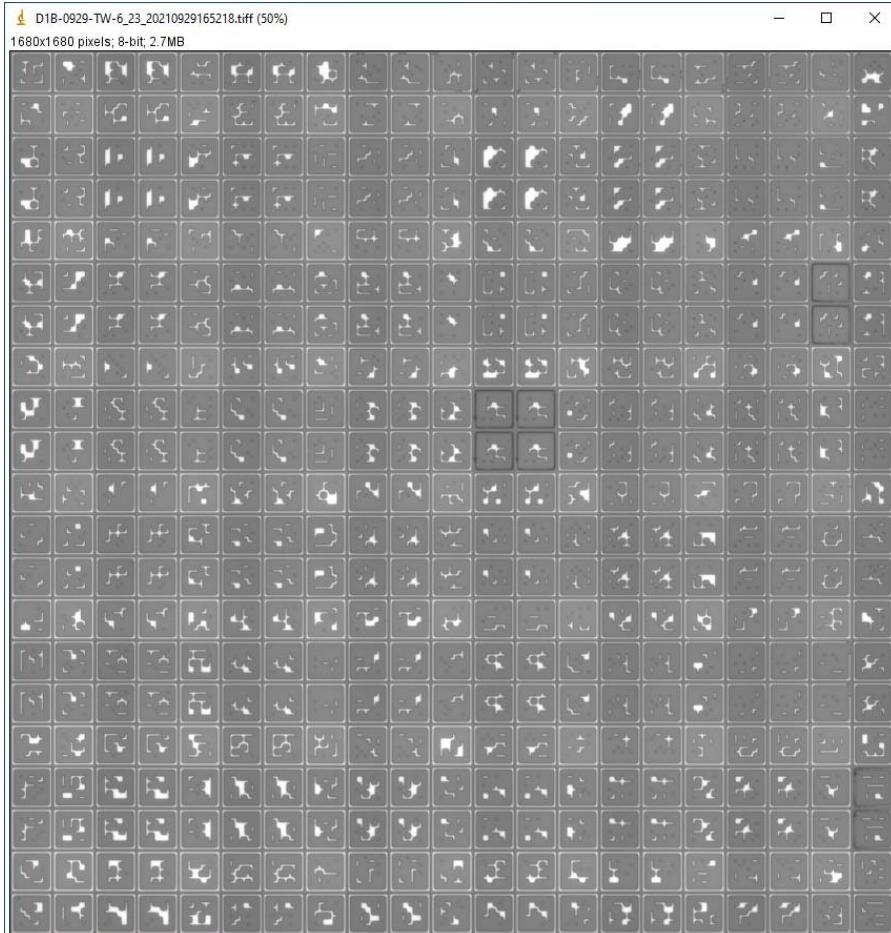
```
// detection parameters
nms_count_ = (fov_wd_ / mk_wd_cl_ + 1) * (fov_hd_ / mk_hd_cl_ + 1);    Alex, 2 years ago • support new aruco recognition ...
nms_radius_ = aruco_marker_->at("nms_radius");
```



# Gridding Development



- Summit.Grid
  - Rescue mechanism for gridding bad fov (for erosion).



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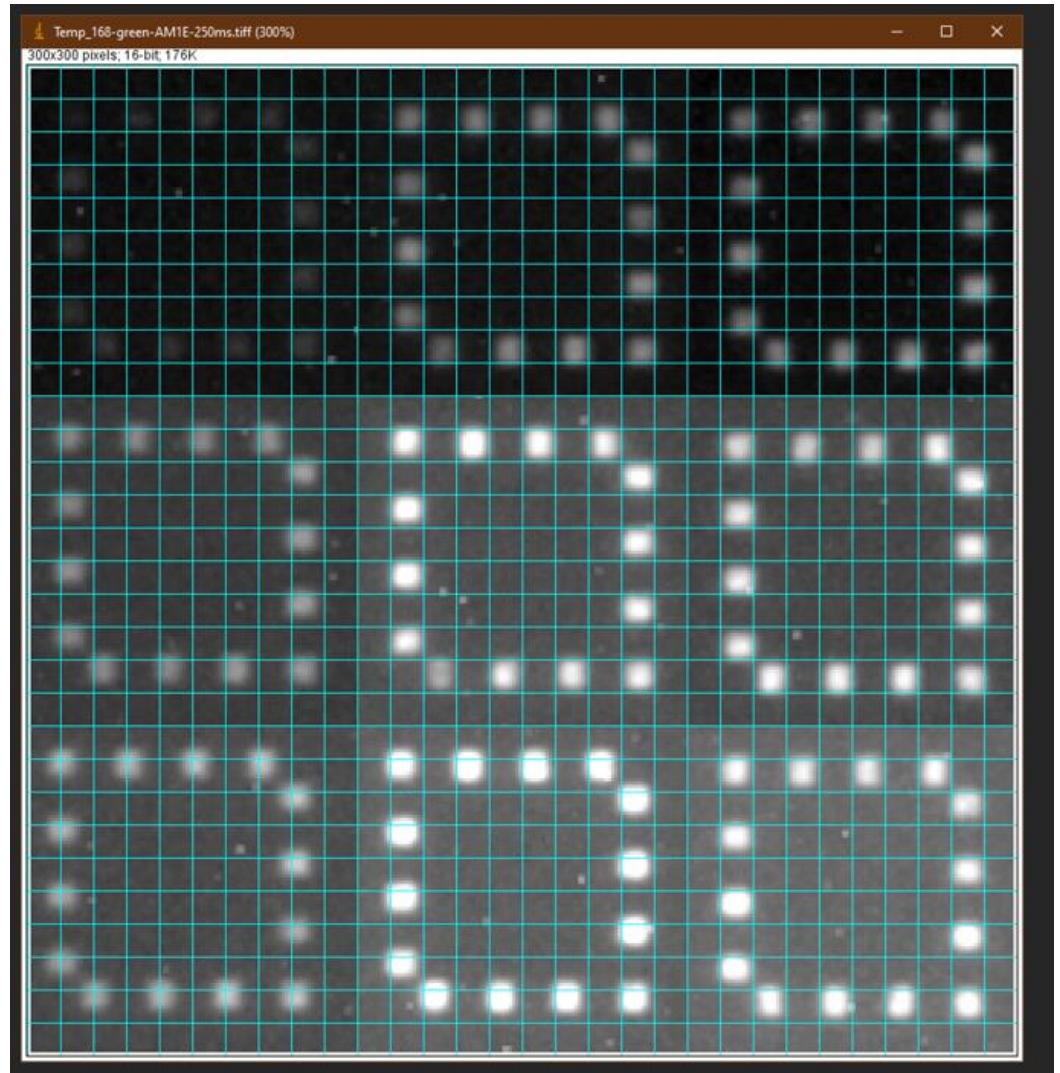
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# Gridding Development



- Summit.Grid
  - PGD Images Processing.

```
],
  "warp_mat": [
    [
      1.3189138576779025,
      0.013670411985018604,
      746.4366977969215
    ],
    [
      -0.013857677902621766,
      1.313483146067416,
      216.52305980929015
    ]
  ]
```



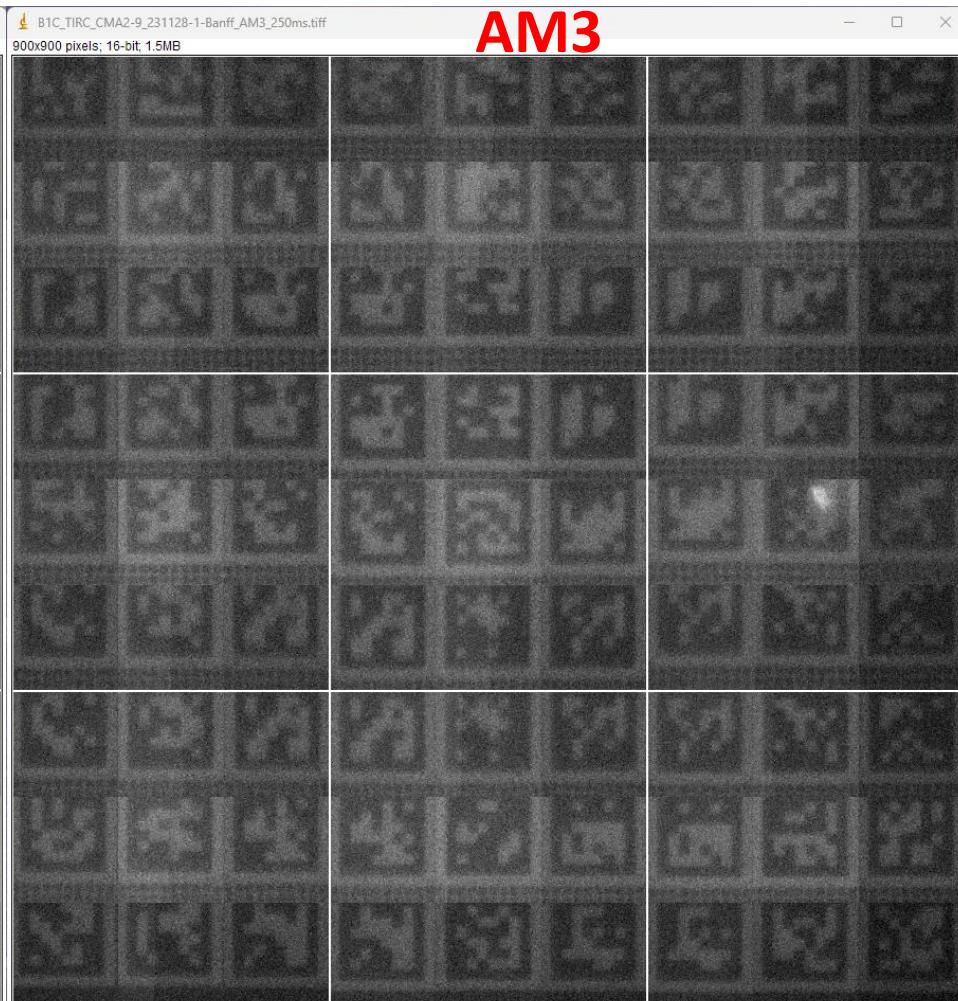
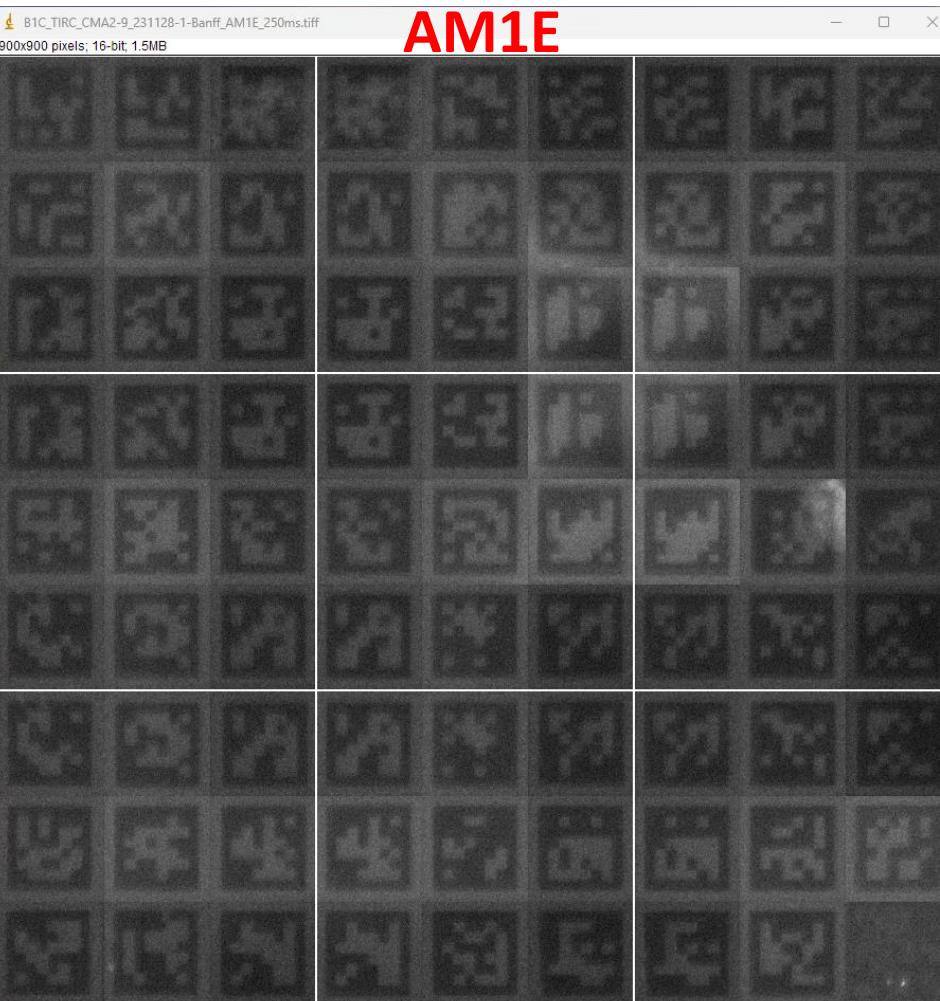
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# Gridding Development



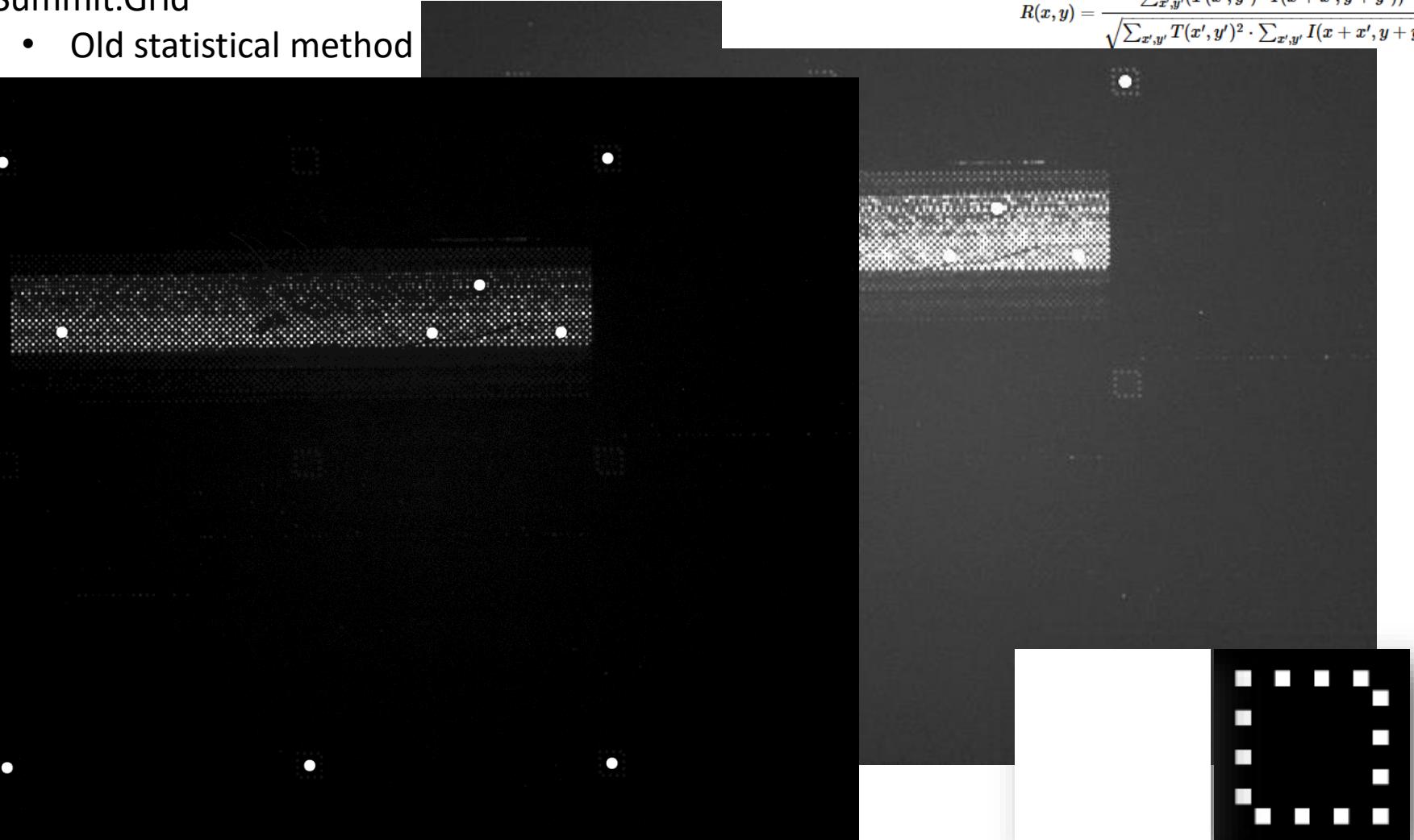
- Summit Grid checking support.



# Performance for New Gridding Software



- Summit.Grid
  - Old statistical method



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# Performance for New Gridding Software



- Summit.Grid
  - New statistical method

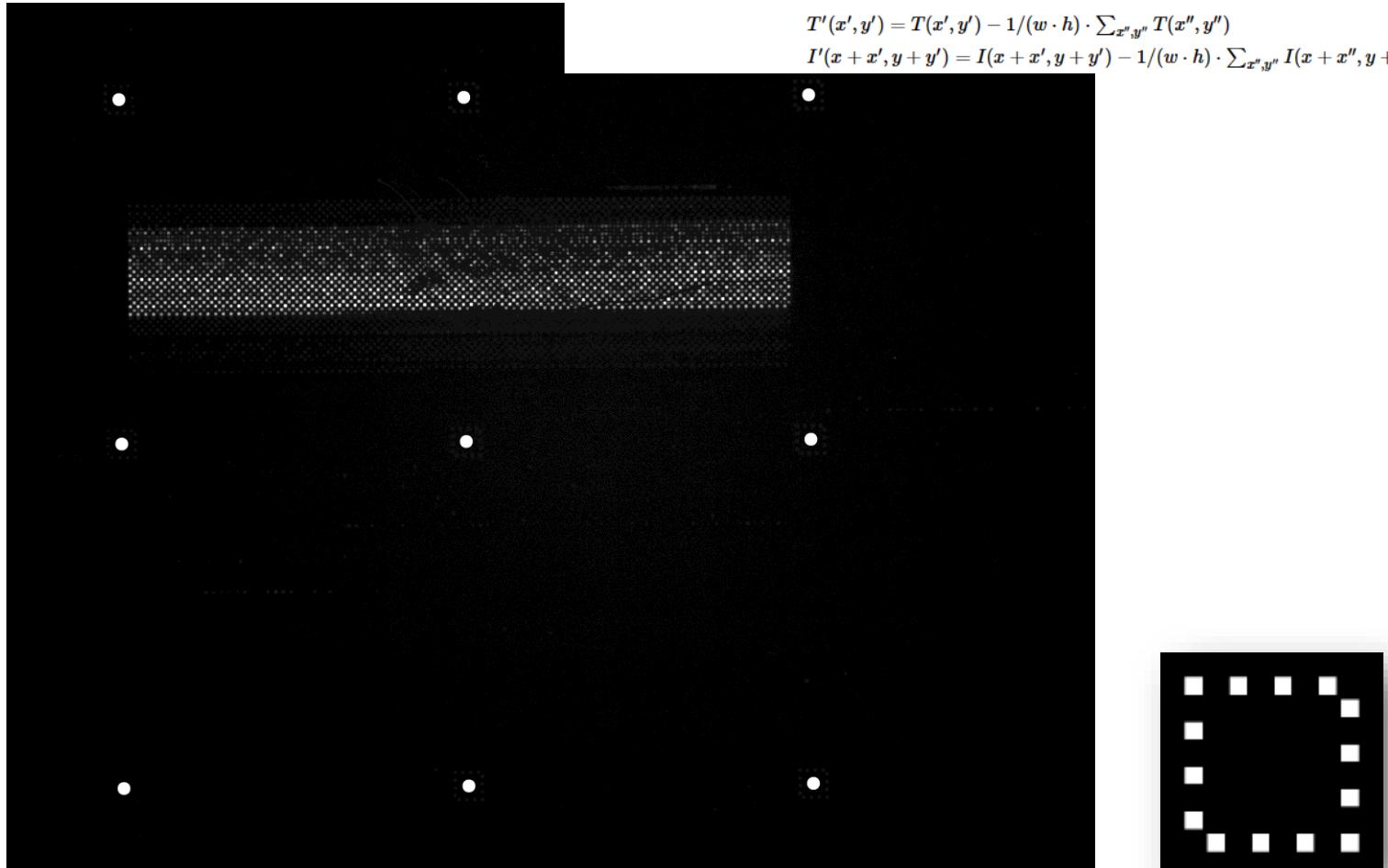
method=TM\_CCOEFF\_NORMED

$$R(x, y) = \frac{\sum_{x',y'}(T'(x', y') \cdot I'(x + x', y + y'))}{\sqrt{\sum_{x',y'} T'(x', y')^2 \cdot \sum_{x',y'} I'(x + x', y + y')^2}}$$

where

$$T'(x', y') = T(x', y') - 1/(w \cdot h) \cdot \sum_{x'',y''} T(x'', y'')$$

$$I'(x + x', y + y') = I(x + x', y + y') - 1/(w \cdot h) \cdot \sum_{x'',y''} I(x + x'', y + y'')$$



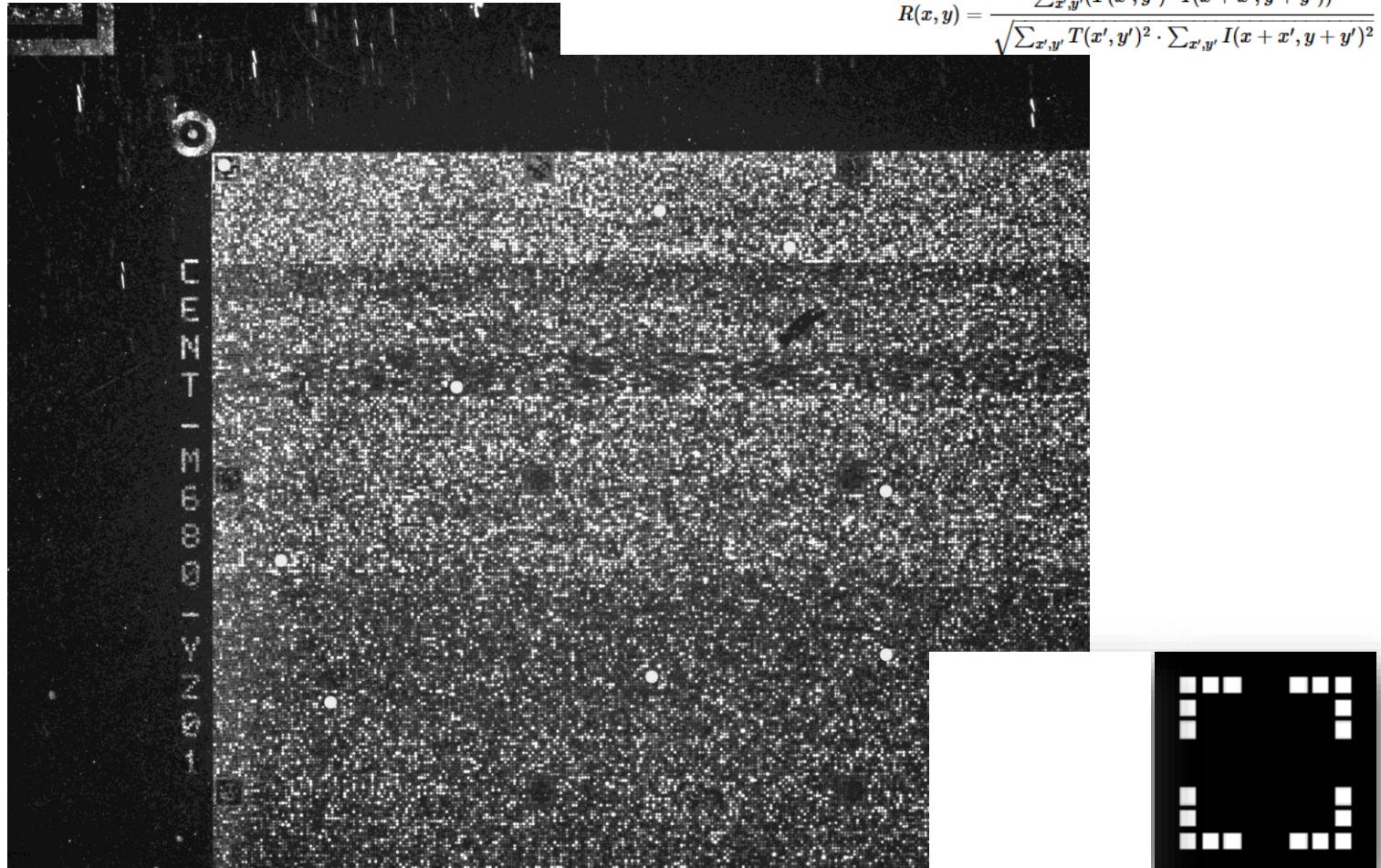
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# Performance for New Gridding Software



- Summit.Grid
  - Old statistical method



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# Performance for New Gridding Software



- Summit.Grid
  - New statistical method



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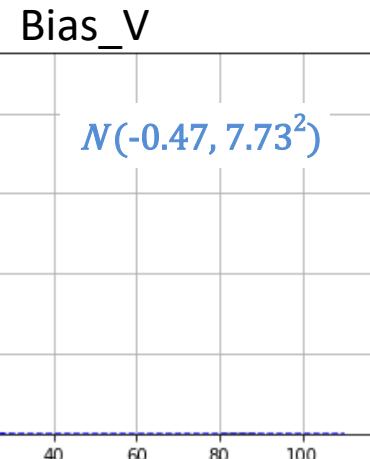
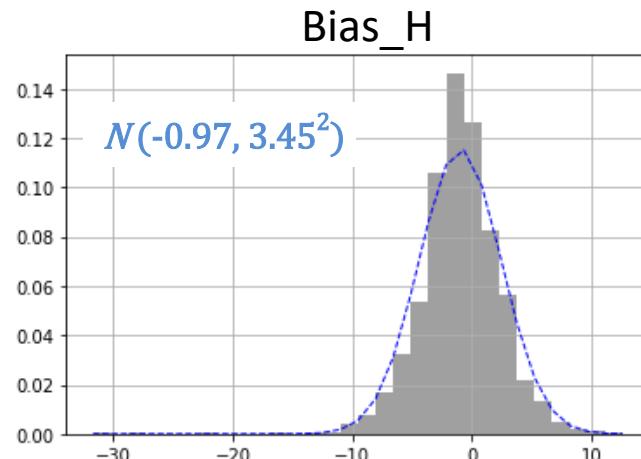
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# Sampling Distribution for Different Chip Scan Mode

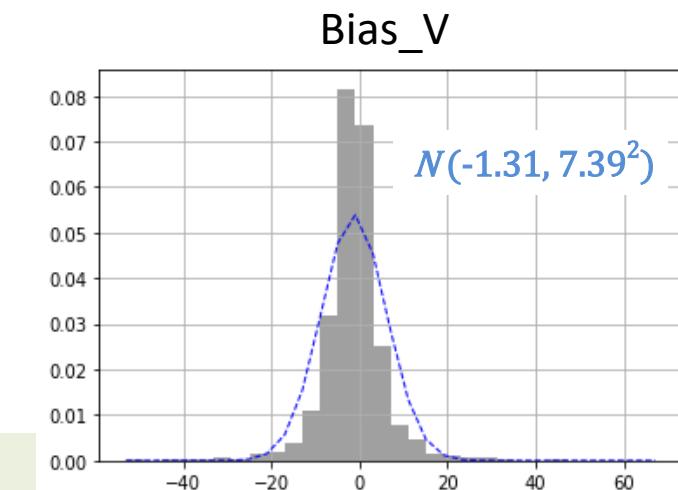
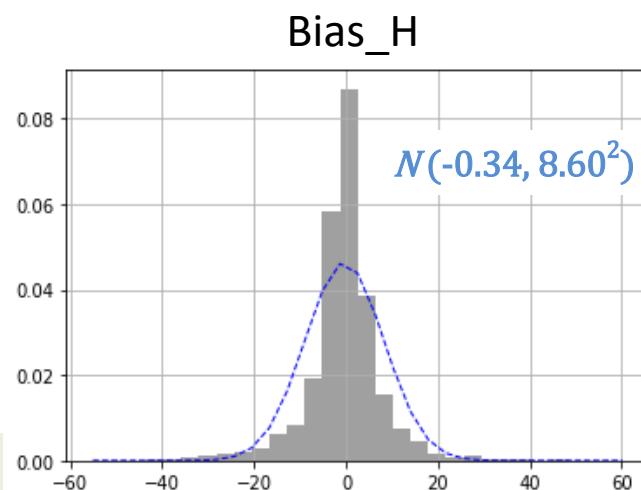


- Quick scan mode experiments
  - Sample: 5 YZ01 chips (7x7 FOVs) x 10 runs => 2450 FOVs
  - Estimation:  $\text{Var}(X+Y)$

SUMMIT Test 2



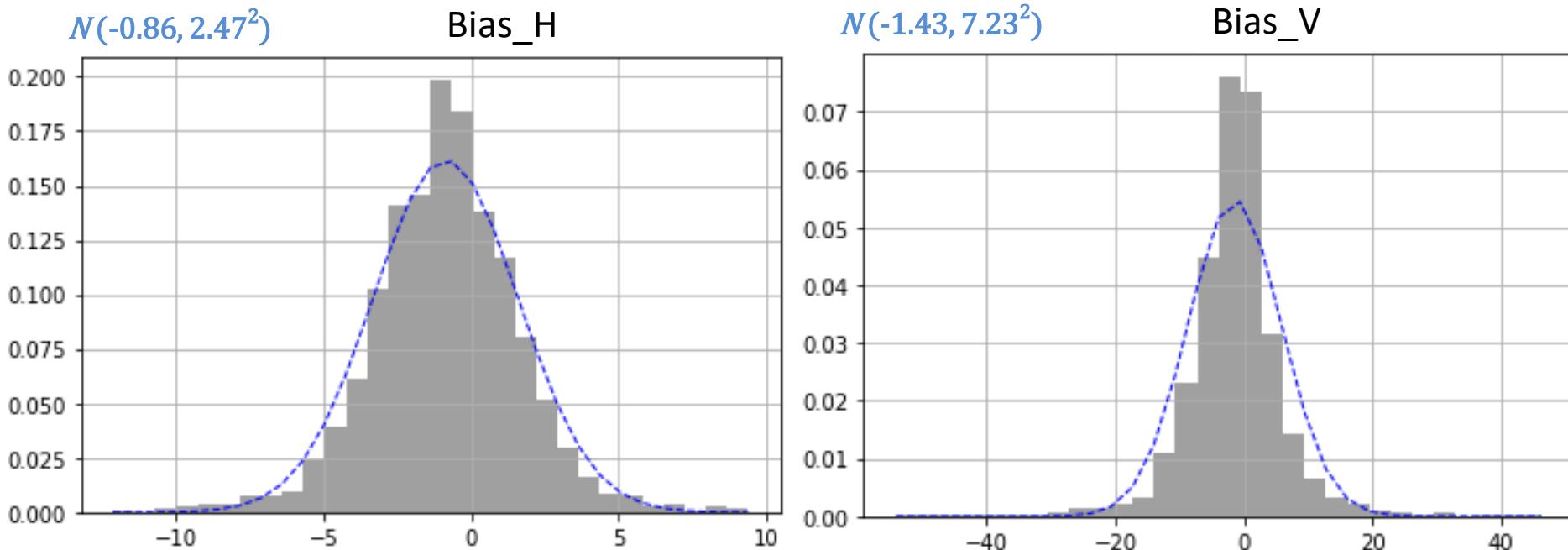
SUMMIT Test 3



# Sampling Distribution for Different Chip Scan Mode



- Quick scan mode experiments
  - Sample: 5 YZ01 chips (7x7 FOVs) x 10 runs => 2450 FOVs
  - Estimation:  $\text{Var}(X+Y)$
  - SUMMIT with precise sliding



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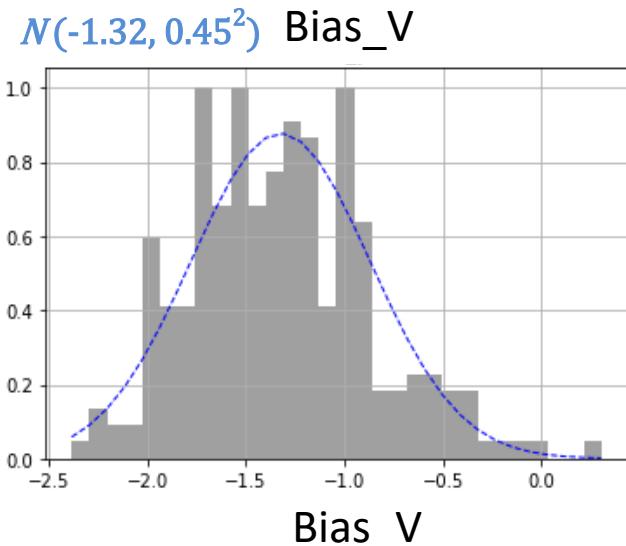
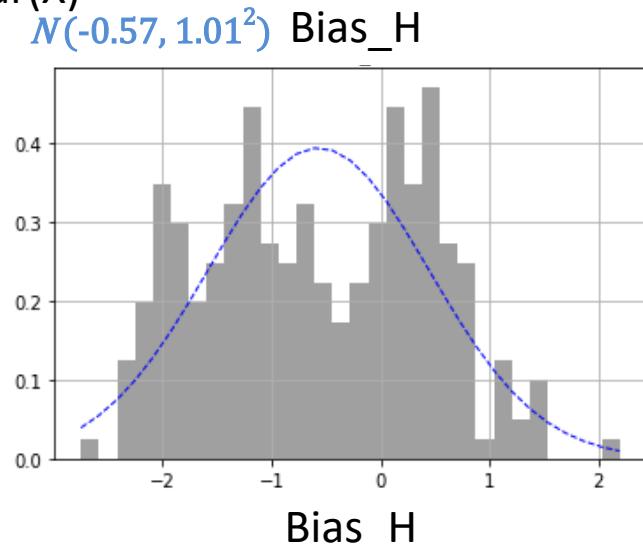
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# Sampling Distribution for Different Chip Scan Mode

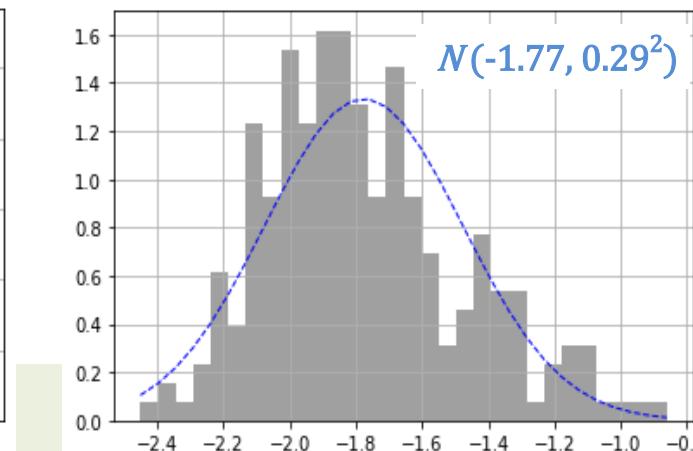
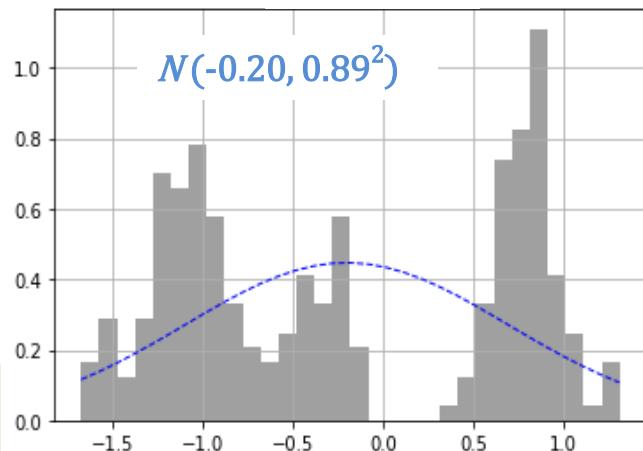


- Regular high precision mode experiments
  - Sample: 5 YZ01 chips (7x7 FOVs) x 1 runs => 245 FOVs
  - Estimation:  $\text{Var}(X)$

SUMMIT Test 2



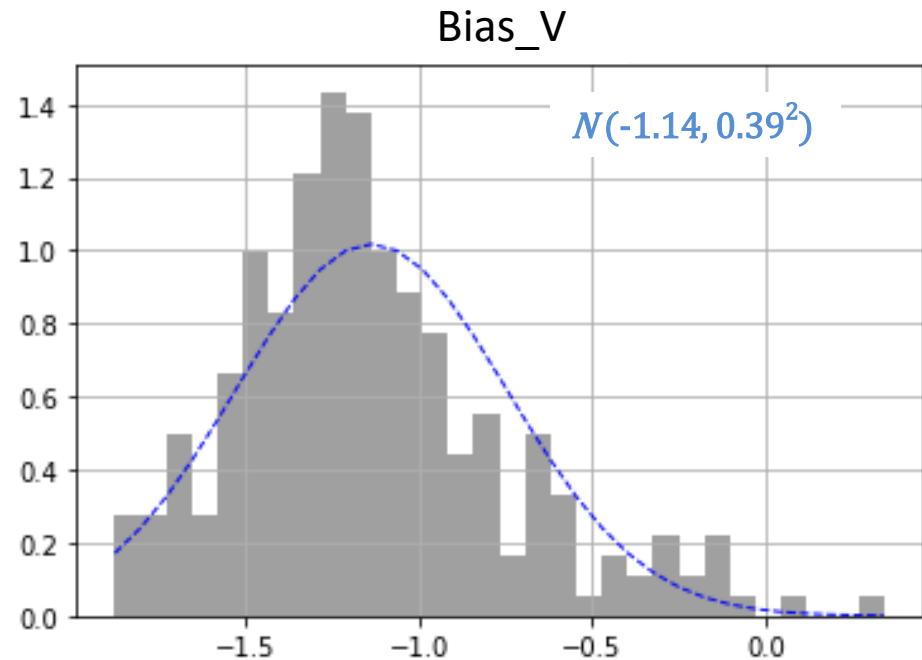
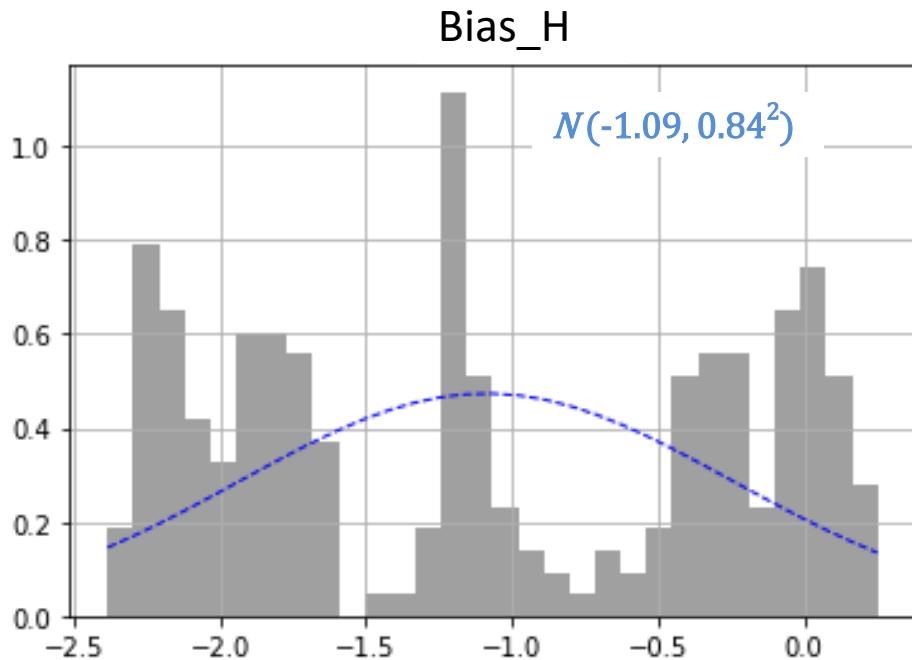
SUMMIT Test 3



# Sampling Distribution for Different Chip Scan Mode



- Regular high precision mode experiments
  - Sample: 5 YZ01 chips (7x7 FOVs) x 1 runs => 245 FOVs
  - Estimation:  $\text{Var}(X)$
  - SUMMIT with Precise Sliding



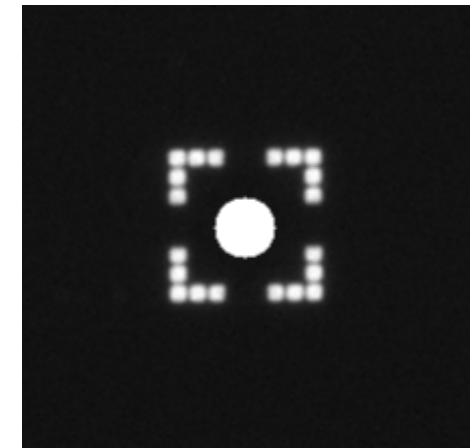
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# Statistical Conclusion from Experiments Results



- Gridding
  - SUMMIT Parameters Estimation
    - Let  $X \equiv r. \nu.$  of the displacement from changing the filter (BF -> fluorescent).
    - Let  $Y \equiv r. \nu.$  of the displacement from relocating the plate to the same position.
    - In the quick scan mode,  
Estimate  $\text{Var}(X + Y) = \text{Var}(X) + \text{Var}(Y)$
    - In the regular high precision mode,  
Estimate  $\text{Var}(X)$  Only



# Statistical Conclusion from Experiments Results



- Gridding
  - Chebyshev's Inequality
    - $P(|Z - \mu| \geq k \cdot \sigma) \leq \frac{1}{k^2}$
    - A. Quick scan for estimating  $Var(X + Y)$ 
      - $k = 14.3, \sigma = 7.74$ , Cover radius: 110.7 (pixels)
      - 99.5% ↑ confidence that the true marker center lies in the cover.
      - 2.3 x BF\_mark\_size, Lower bound: 110.7 (pixels)
    - B. Regular scan for estimating  $Var(X)$ 
      - $k = 7.2, \sigma = 1.01$ , Cover radius: 7.272 (pixels)
      - 98.06% ↑ confidence that the true marker center lies in the cover.
      - 0.15 x BF\_mark\_size
  - Normal Distribution
    - $P(|T - \mu| < Z_{0.0025} \cdot \sigma) = 99.5\%, Z_{0.0025} = 2.807$ , 99.5% confidence that the true marker center lies in the cover.
    - A. Quick scan for estimating  $Var(X + Y)$ 
      - $\sigma = 7.74$ , Cover radius: 21.7 (pixels)
    - B. Regular scan for estimating  $Var(X)$ 
      - $\sigma = 1.01$ , Cover radius: 2.8 (pixels)
  - Overall Performance - successfully recognized rate: nearly 100%.

# Signal Intensity Extraction Techniques Comparison



- Raw Data (NPcall Analyzer)

Grid2 (current)	No.	Data	Grid1	Grid2_subpix	Grid2_subpix_cvfix	Ranking		
85.245%	1	85_46_20210324123447	85.944%	86.131%	86.131%	3	1	1
86.113%	2	85_68_20210303140400	87.582%	87.506%	87.506%	1	2	2
84.400%	3	85_69_20210303142117	87.605%	87.261%	87.261%	1	2	2
85.746%	4	85_76_20210303143808	86.719%	87.244%	87.238%	3	1	2
87.552%	5	85_77_20210303145506	87.751%	88.974%	88.817%	3	1	2
93.293%	6	90_21_20210325132601	93.916%	93.893%	93.945%	2	3	1
92.110%	7	90_54_20210324125308	92.512%	92.587%	92.634%	3	2	1
92.255%	8	90_62_20210324131130	93.036%	92.978%	92.984%	1	3	2
91.748%	9	90_70_20210324132953	92.657%	92.611%	92.611%	1	2	2
90.653%	10	90_77_20210325142116	91.492%	91.533%	91.550%	3	2	1
93.467%	11	95_38_20210324121430	94.015%	94.079%	94.161%	3	2	1
95.798%	12	95_76_20210324164230	96.096%	96.294%	96.311%	3	2	1
94.837%	13	95_77_20210221134715_94_8	95.402%	95.367%	95.379%	1	3	2
95.868%	14	95_77_20210324165927	96.230%	96.317%	96.270%	3	1	2
94.965%	15	95_78_20210221140401	95.688%	95.641%	95.624%	1	2	3

# Signal Intensity Extraction Techniques Comparison



- Raw Data (GT Caller)

No.	Data	Grid1	Grid2_subpix	Grid2_subpix_cvfix	Ranking		
1	85_46_20210324123447	92.225%	93.225%	93.358%	3	2	1
2	85_68_20210303140400	93.333%	92.650%	92.450%	1	2	3
3	85_69_20210303142117	93.325%	92.991%	92.958%	1	2	3
4	85_76_20210303143808	92.492%	93.266%	93.050%	3	1	2
5	85_77_20210303145506	93.058%	94.208%	94.192%	3	1	2
6	90_21_20210325132601	98.667%	99.600%	98.825%	3	1	2
7	90_54_20210324125308	98.175%	98.369%	98.183%	3	1	2
8	90_62_20210324131130	98.500%	98.392%	98.458%	1	3	2
9	90_70_20210324132953	98.108%	97.942%	97.950%	1	3	2
10	90_77_20210325142116	97.950%	98.158%	98.158%	3	1	1
11	95_38_20210324121430	99.092%	99.008%	99.025%	1	3	2
12	95_76_20210324164230	99.666%	99.666%	99.683%	2	2	1
13	95_77_20210221134715_94_8	99.392%	99.416%	99.408%	3	1	2
14	95_77_20210324165927	99.583%	99.608%	99.608%	3	1	1
15	95_78_20210221140401	99.367%	99.316%	99.333%	1	3	2

# Signal Intensity Extraction Techniques Comparison



- Multiple data NP call results comparison (from finally 38 results)
  - $H_0$ : Grid1 intensity  $\geq$  Intensity extracted from new developed process (Grid2)
  - $H_1$ : Grid1 intensity  $<$  Intensity extracted from new developed process (Grid2)

t-Test: Paired Two sample for Means (NP call Analyzer)

	Grid1	Grid2_subpix
Mean	15307.82185	15322.24077
Variance	2060861.144	2050166.456
Observations	38	38
Pearson Correlation	0.999513654	
Hypothesized Mean Difference	0	
Df	37	
t Stat	-1.980941596	
P(T<=t) one-tail	0.027533779	
t Critical one-tail	1.68709362	
P(T<=t) two-tail	0.055067559	
t Critical two-tail	2.026192463	

	Grid1	Grid2_subpix_cvfix
Mean	15307.82185	15322.34463
Variance	2060861.144	2047607.206
Observations	38	38
Pearson Correlation	0.999572526	
Hypothesized Mean Difference	0	
df	37	
t Stat	-2.123345038	
P(T<=t) one-tail	0.020238129	
t Critical one-tail	1.68709362	
P(T<=t) two-tail	0.040476259	
t Critical two-tail	2.026192463	

⇒ Reject  $H_0$

t-Test: Paired Two sample for Means (GT Caller)

	Grid1	Grid2_subpix
Mean	16100.97195	16126.25089
Variance	2272377.702	2210252.078
Observations	38	38
Pearson Correlation	0.998958931	
Hypothesized Mean Difference	0	
df	37	
t Stat	-2.182738079	
P(T<=t) one-tail	0.017738267	
t Critical one-tail	1.68709362	
P(T<=t) two-tail	0.035476534	
t Critical two-tail	2.026192463	

	Grid1	Grid2_subpix_cvfix
Mean	16100.97195	16120.53029
Variance	2272377.702	2195827.937
Observations	38	38
Pearson Correlation	0.99898407	
Hypothesized Mean Difference	0	
df	37	
t Stat	-1.672830702	
P(T<=t) one-tail	0.051398391	
t Critical one-tail	1.68709362	
P(T<=t) two-tail	0.102796783	
t Critical two-tail	2.026192463	

⇒ Reject  $H_0$



# **Normal Gamma Background Correction & Data Preprocess**

Jeff (CHI-HSUAN HO)

- **Model Assumption**

- For each single array:

$$\textcolor{green}{X}_j = \textcolor{orange}{S}_j + \textcolor{blue}{B}_j$$

- $BgC : \textcolor{green}{X}_j \Rightarrow \textcolor{orange}{S}_j$  Enhance the biological validity of the results.

---

**Improving background correction for Illumina BeadArrays: the normal-gamma model.**

Sandra Plancade <sup>1\*</sup>, Yves Rozenholc <sup>2</sup>, Eiliv Lund <sup>1</sup>

<sup>1</sup>Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, 9037 Tromsø, Norway.

<sup>2</sup>Department of Applied Mathematics, MAP5, 45 rue des Saints-Pères, University Paris Descartes, 75006 Paris.

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**ABSTRACT**

**Motivation:** Illumina beadarray technology provides high quality data, including non specific negative control features which allow a precise estimation of the background noise. As reported in many studies, the traditional background subtraction proposed in BeadStudio leads

Namely, let  $X$  be the observed intensity of a given probe, we assume that

$$X = S + B \quad (1)$$

where  $S$  is the true signal which counts for the abundance of

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- **Models and Notations**

- For each single array  $j$ :

$$\textcolor{teal}{X}_j = \textcolor{orange}{S}_j + \textcolor{blue}{B}_j$$

- $X_j = \begin{cases} S_j + B_j, & j \in J \Rightarrow \text{regular probes set} \\ 0 + B_j = B_j, & j \in J_0 \Rightarrow \text{negative control probes set} \end{cases}$
- $\textcolor{teal}{X}_j \sim f_x(x)$ ,  $\textcolor{orange}{S}_j \sim f_s(s)$ ,  $\textcolor{blue}{B}_j \sim f_B(b)$ ,  $\textcolor{orange}{S}_j$  and  $\textcolor{blue}{B}_j$  are independent.
- $N(\mu, \sigma^2) \Rightarrow f_{\mu, \sigma}^{\text{norm}}(x) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left\{-\frac{(x-\mu)^2}{2\sigma^2}\right\}$
- $\phi(x) \Rightarrow N(0, 1)$ ,  $\Phi(t) = \int_{-\infty}^t \phi(x) dx$
- $\text{Gamma}(k, \theta) \Rightarrow f_{k, \theta}^{\text{gam}}(x) = \frac{\left(\frac{1}{\theta}\right)^k}{\Gamma(k)} x^{k-1} \exp\left\{-\frac{x}{\theta}\right\}$ ,  $k$ : shape parameter,  $\theta$ : scale parameter  
 $\xrightarrow[k=1, \theta=\alpha]{} \text{Exp}(\alpha) \Rightarrow f_{\alpha}^{\text{exp}}(x) = \frac{1}{\alpha} \exp\left\{-\frac{x}{\alpha}\right\}$

- **Models and Notations**

- $X_j = S_j + B_j, \quad X_j \sim f_x(x), \quad S_j \sim f_s(s), \quad B_j \sim f_B(b)$
- By the convolution formula,  $x_j = s_j + b_j \Rightarrow b_j = x_j - s_j \Rightarrow |J| = \left| \frac{db_j}{dx_j} \right| = 1$   
 $\Rightarrow X_j \sim f_x(x) = \int_{-\infty}^{\infty} f_{X,S}(x,s) ds = \int_{-\infty}^{\infty} f_{S,B}(s, x-s) |J| ds = \int_{-\infty}^{\infty} f_{S,B}(s, x-s) ds$   
 $= \int_{-\infty}^{\infty} f_s(s) f_B(x-s) ds$   
 $\Rightarrow$  Estimated Signal:  $\hat{S}(x) = E[S|X=x] = \int_{-\infty}^{\infty} S f_{S|X=x}(s) ds = \int_{-\infty}^{\infty} S \frac{f_{S,X}(s,x)}{f_x(x)} ds$   
 $= \frac{\int_{-\infty}^{\infty} S f_{S,X}(s,x) ds}{\int_{-\infty}^{\infty} f_{S,X}(s,x) ds} = \frac{\int_{-\infty}^{\infty} S f_s(s) f_B(x-s) ds}{\int_{-\infty}^{\infty} f_s(s) f_B(x-s) ds}$
- Thus, if  $f_x(x)$  is known  $\Rightarrow \hat{S}(x)$  is known.
- No analytic expression  $\Rightarrow$  Fast Fourier Transformation-based (fft) approximation.

- **The normexp Model**

- $S_j \sim f_s(s) = \begin{cases} Exp(\alpha), & j \in J \\ 0, & j \in J_0 \end{cases}, \quad B_j \sim f_B(b) \Rightarrow N(\mu, \sigma^2)$

$$\Rightarrow X_j \sim f_X(x) \equiv f_{\mu, \sigma, \alpha}^{nexp}(x) = \frac{1}{\alpha} \exp\left\{\frac{\sigma^2}{2\alpha^2} - \frac{x-\mu}{\alpha}\right\} \Phi(\bar{x}), \quad \text{where } \bar{x} = \frac{(x-\mu-\frac{\sigma^2}{\alpha})}{\sigma}$$
$$\Rightarrow \hat{S}^{nexp}(x|\Theta) = \sigma\left(\bar{x} + \frac{\phi(\bar{x})}{\Phi(\bar{x})}\right), \quad \Theta = (\mu, \sigma, \alpha)$$

- If we know  $(\hat{\mu}, \hat{\sigma}, \hat{\alpha}) \Rightarrow$  we know  $\hat{S}^{nexp}(x)$

- **The Parameter Estimation of normexp Model**

- MLE
- Adapted RMA
- Non-parametric estimation (NP)
- Bayesian estimation

- **The normal-gamma Model**

- $S_j \sim f_s(s) = \begin{cases} \text{Gamma}(k, \theta), & j \in J \\ 0, & j \in J_0 \end{cases}, B_j \sim f_B(b) \Rightarrow N(\mu, \sigma^2)$   
 $\Rightarrow X_j \sim f_X(x) \equiv f_{\mu, \sigma, k, \theta}^{ng}(x) = \int f_{k, \theta}^{gam}(t) f_{\mu, \sigma}^{norm}(x - t) dt \Rightarrow fft-based approximation$   
 $\Rightarrow \hat{S}^{ng}(x|\Theta) = \frac{\int s f_{k, \theta}^{gam}(s) f_{\mu, \sigma}^{norm}(x-s) ds}{f_{\mu, \sigma, k, \theta}^{ng}(x)} = \frac{k\theta \left( \int f_{k+1, \theta}^{gam}(s) f_{\mu, \sigma}^{norm}(x-s) ds \right)}{f_{\mu, \sigma, k, \theta}^{ng}(x)}$   
 $= \frac{k\theta f_{\mu, \sigma, k+1, \theta}^{ng}(x)}{f_{\mu, \sigma, k, \theta}^{ng}(x)} \Rightarrow fft-based approximation$
- If we know  $(\hat{\mu}, \hat{\sigma}, \hat{k}, \hat{\theta}) \Rightarrow$  we know  $\hat{S}^{ng}(x) \Rightarrow \hat{S}_j = \hat{S}^{ng}(x_j)$

- **The Parameter Estimation of normal-gamma Model**

A. MLE with classical minimization algorithms (L-BFGS-B)

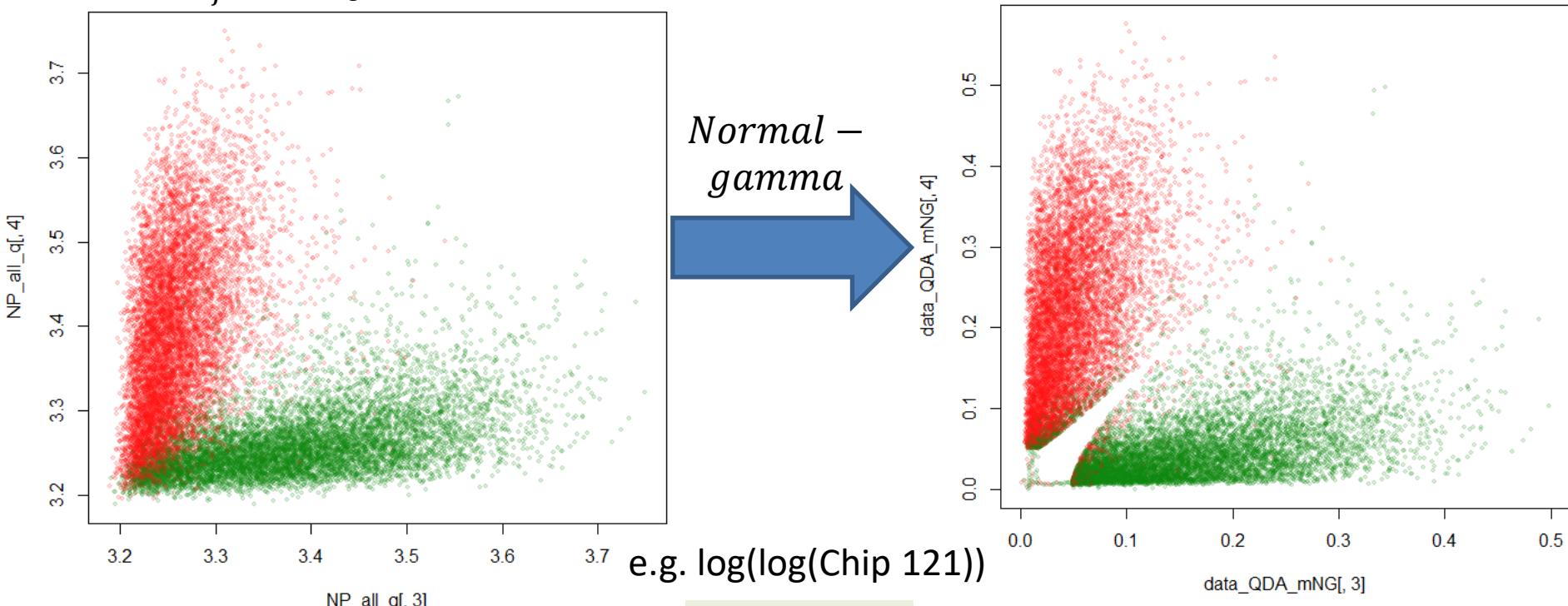
# Performance on the Real Data

- Normal-gamma BgC.

- For each single probe in each cluster (channel):

$$X_j = S_j + N_j$$

- Normal – gamma* Correction:  $X_j \Rightarrow S_j$ . Enhance the biological validity of the results.
- $N_j$  represents the noise.  $\Rightarrow$  Assumption: Normal distribution.
- Remove  $N_j$  and use Gamma distribution to estimate  $S_j$ .
- $X_j \Rightarrow$  Normal-gamma distribution.



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# Performance on the Real Data



- GMM-EM + Normal-gamma BgC.
  - Set the related environment in R. (Data Preprocess, NP Probes, QN & log, QDA)
  - Set the corresponding evaluation tools in R. (NP call rate, No call rate)
  - Run the GMM-EM in R. (10 times => max NP call.)
  - Run the normal-gamma correction (all together) before running QDA.
  - Debug for the no call rate in the NP call analyzer.
  - An example: NP call Analyzer: NP call: 94.4%, call: 66.9%

NP call ( $\log(\log(\cdot))$ )	
GMM + EM	94.2308%
QDA	94.5863%
ALL_NG + QDA	94.2774%

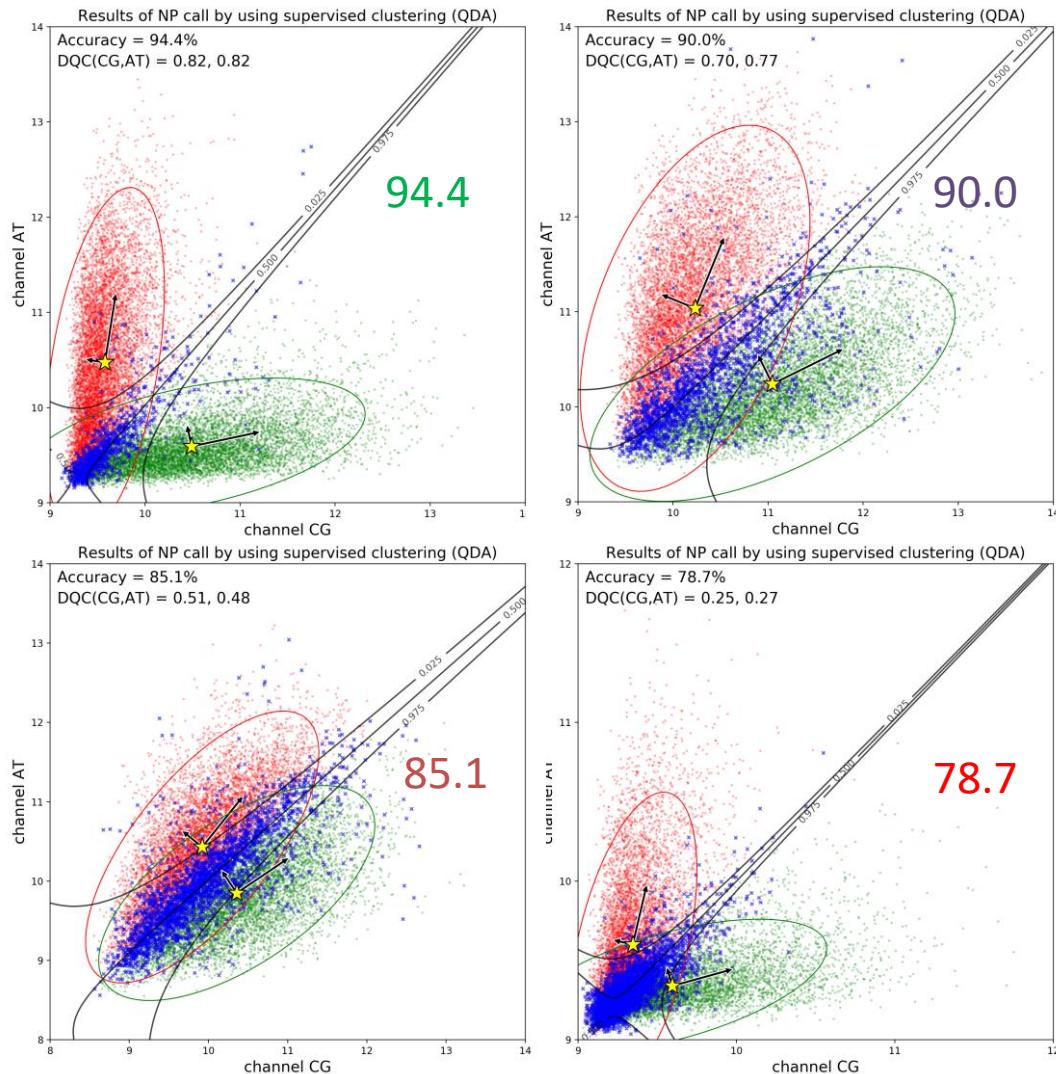
Call rate ( $\log(\log(\cdot))$ )	
GMM + EM	66.8298%
QDA	67.8205%
ALL_NG + QDA	67.7681%

# Data Preprocess



- Data Example

Wafer	Chips	Np call rate (%) (NP call Analyzer)
198-04	121	94.4
197-02	230	90.0
198-15	277	85.1
197-02	233	78.7



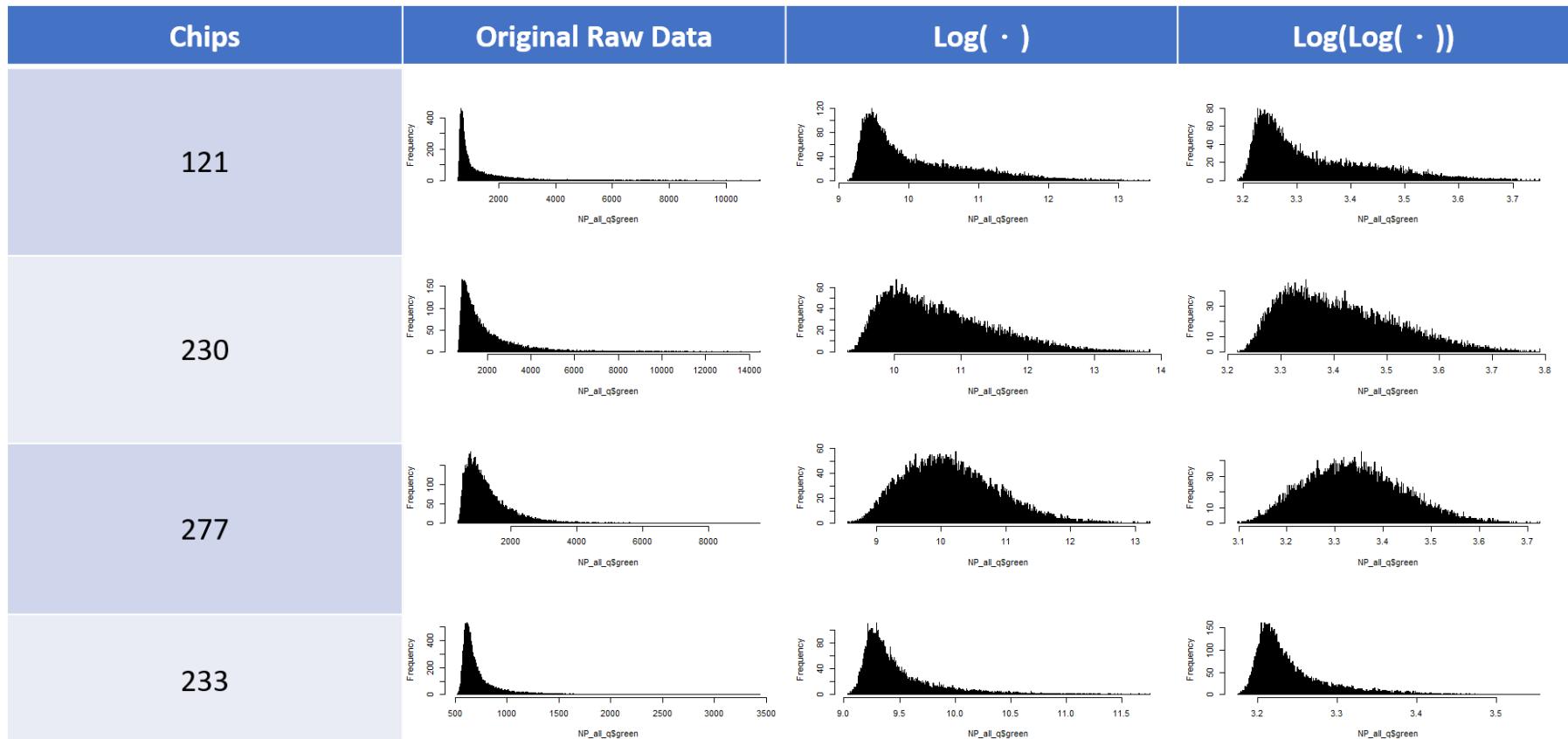
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# Data Preprocess



- Logarithm Effect



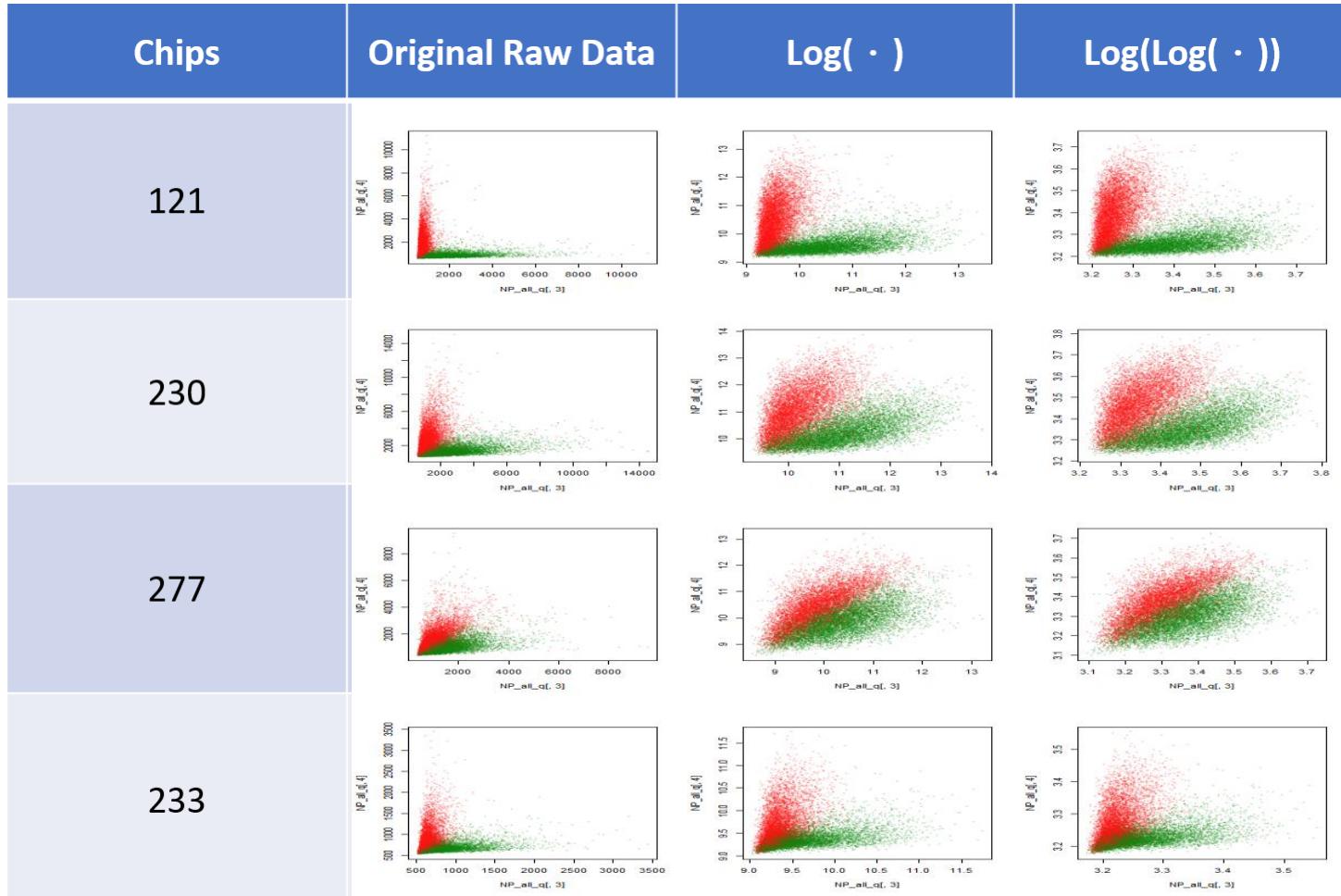
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# Data Preprocess



- Logarithm Effect



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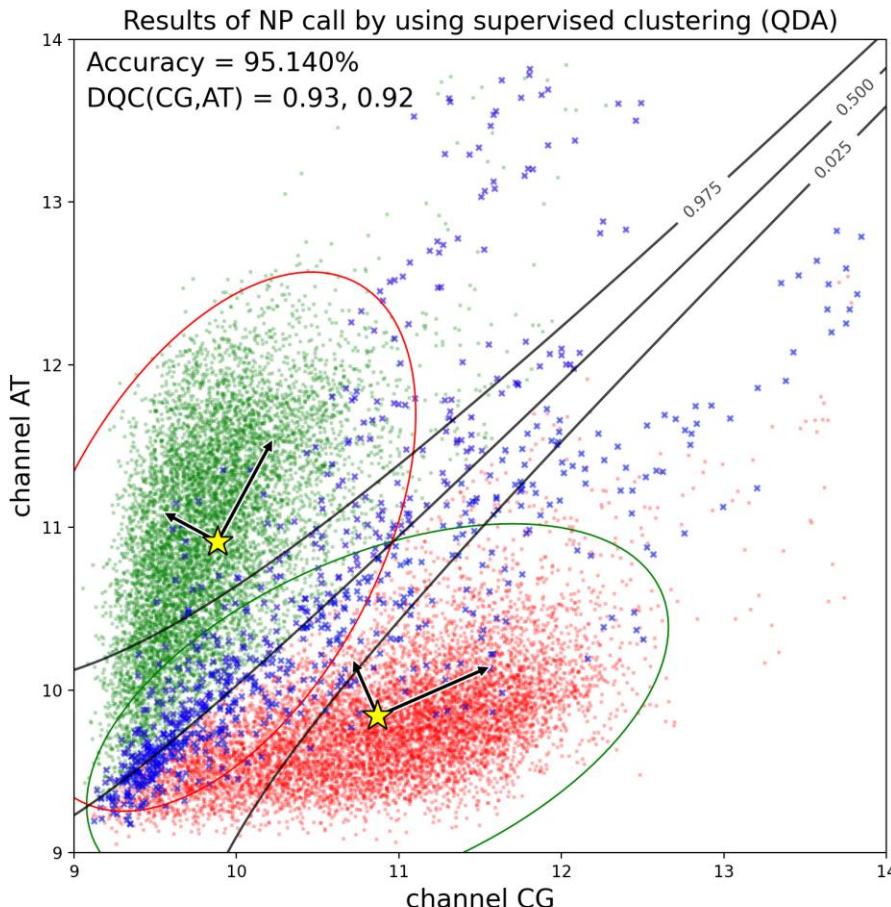
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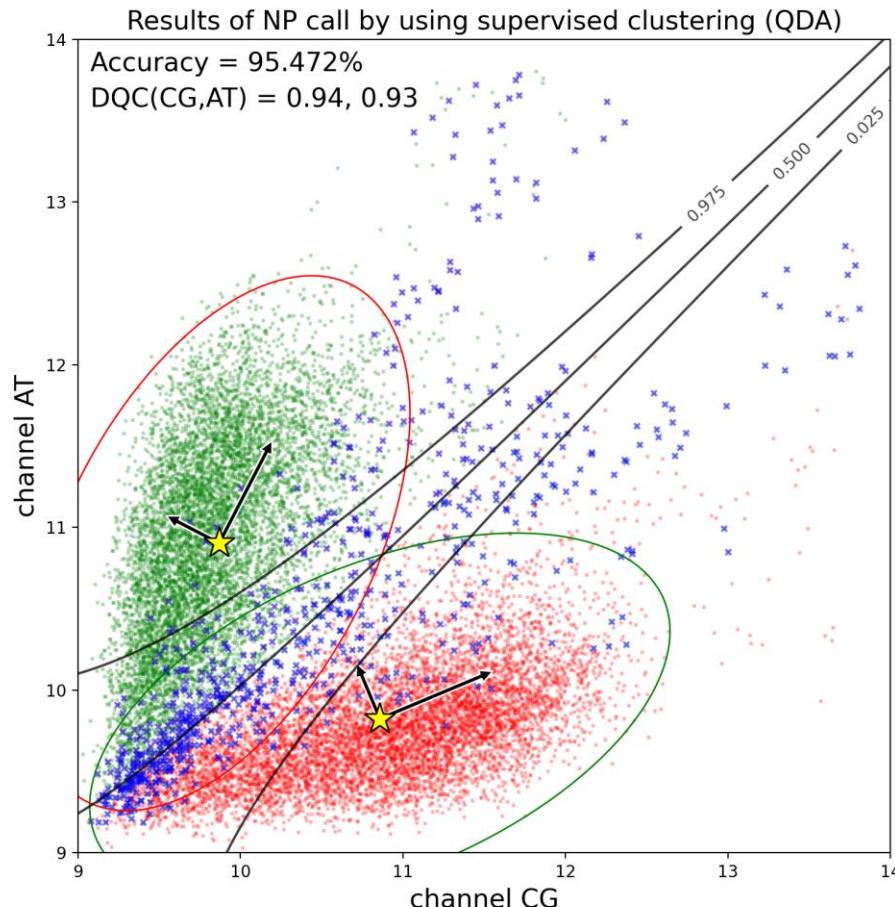
# Chip QC and NPcall Analyzer

Jeff (CHI-HSUAN HO)

## Chip No.54 Quality Control

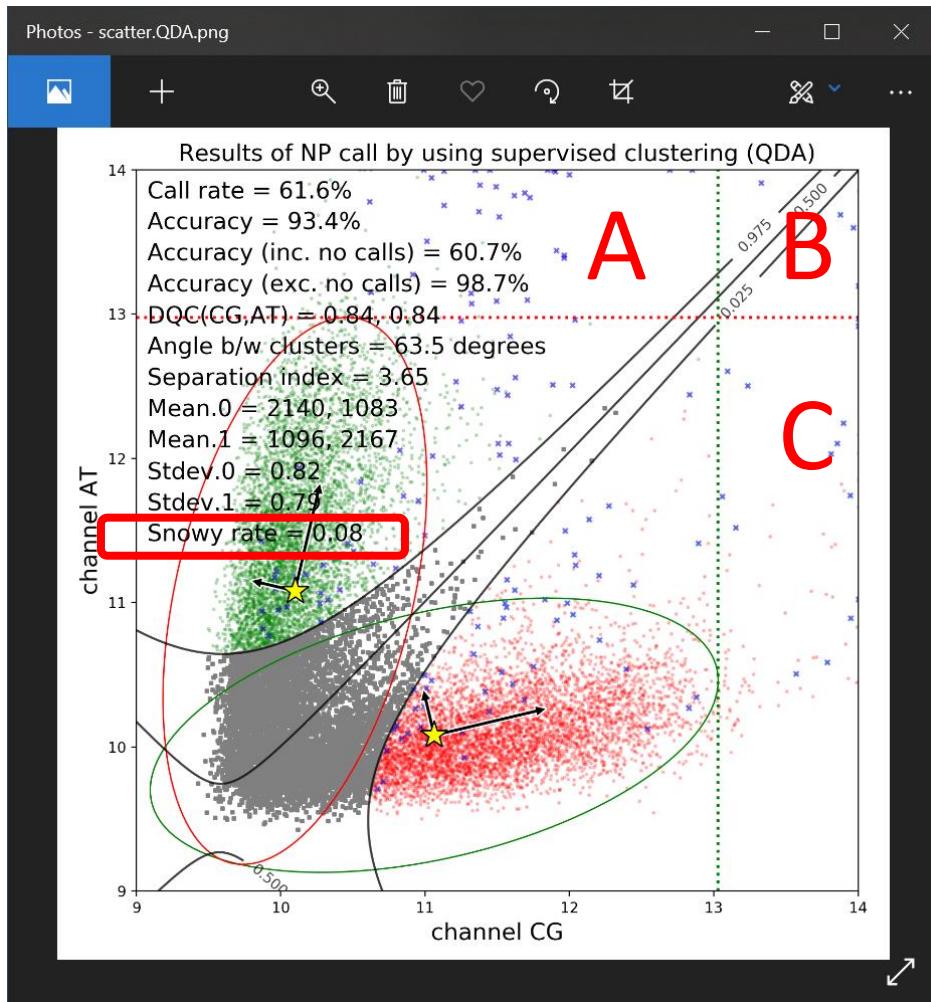


## Chip NO.62 Quality Control



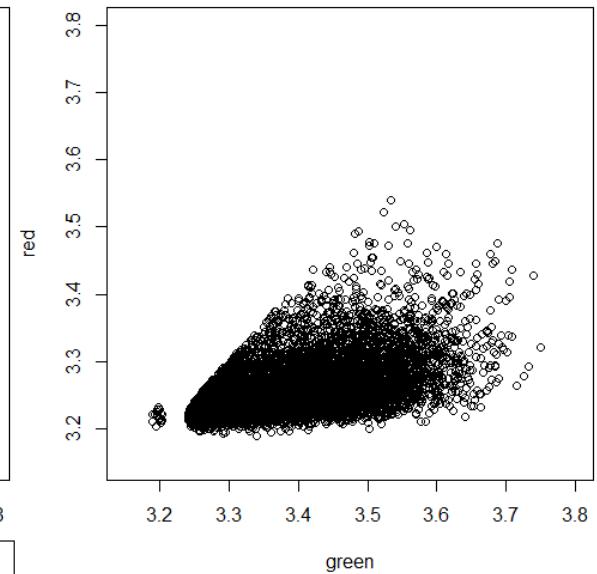
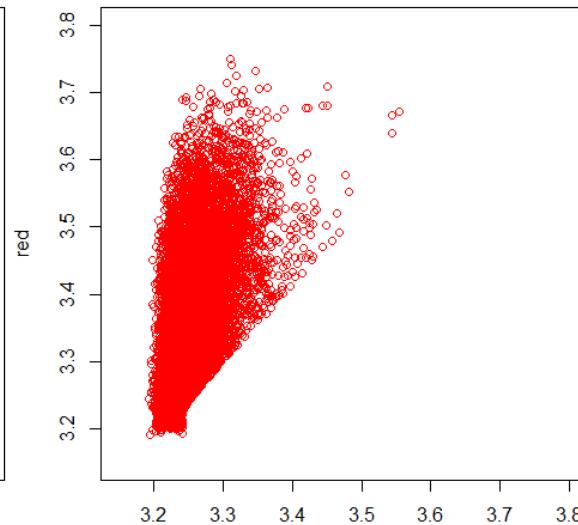
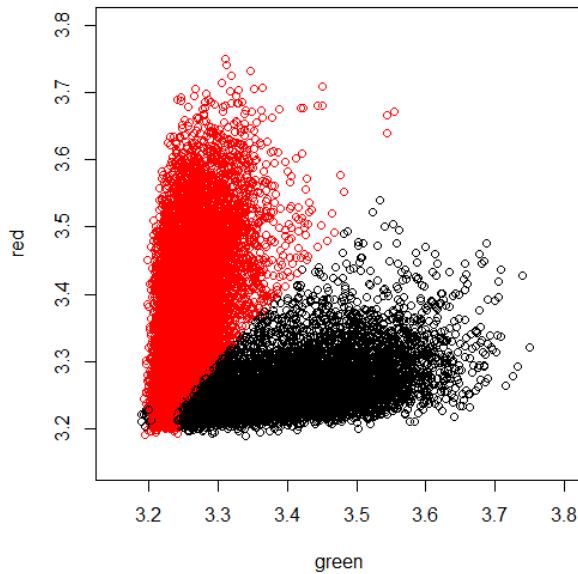
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- Snowy rate =  $\frac{x \text{ in } B}{\text{all points in } A+B+C}$

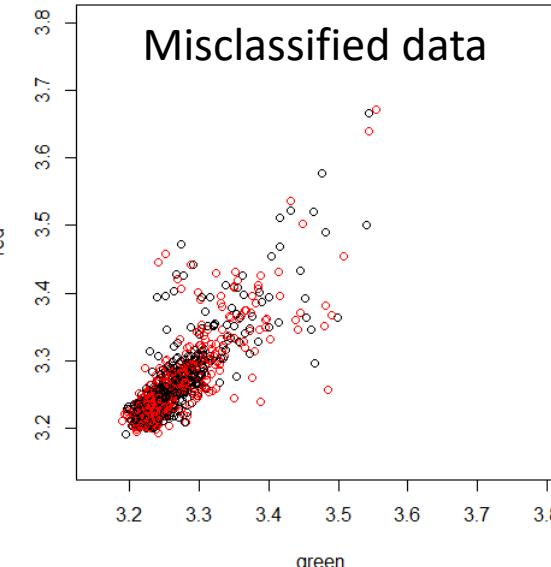
- GMM-EM Results (log(log))



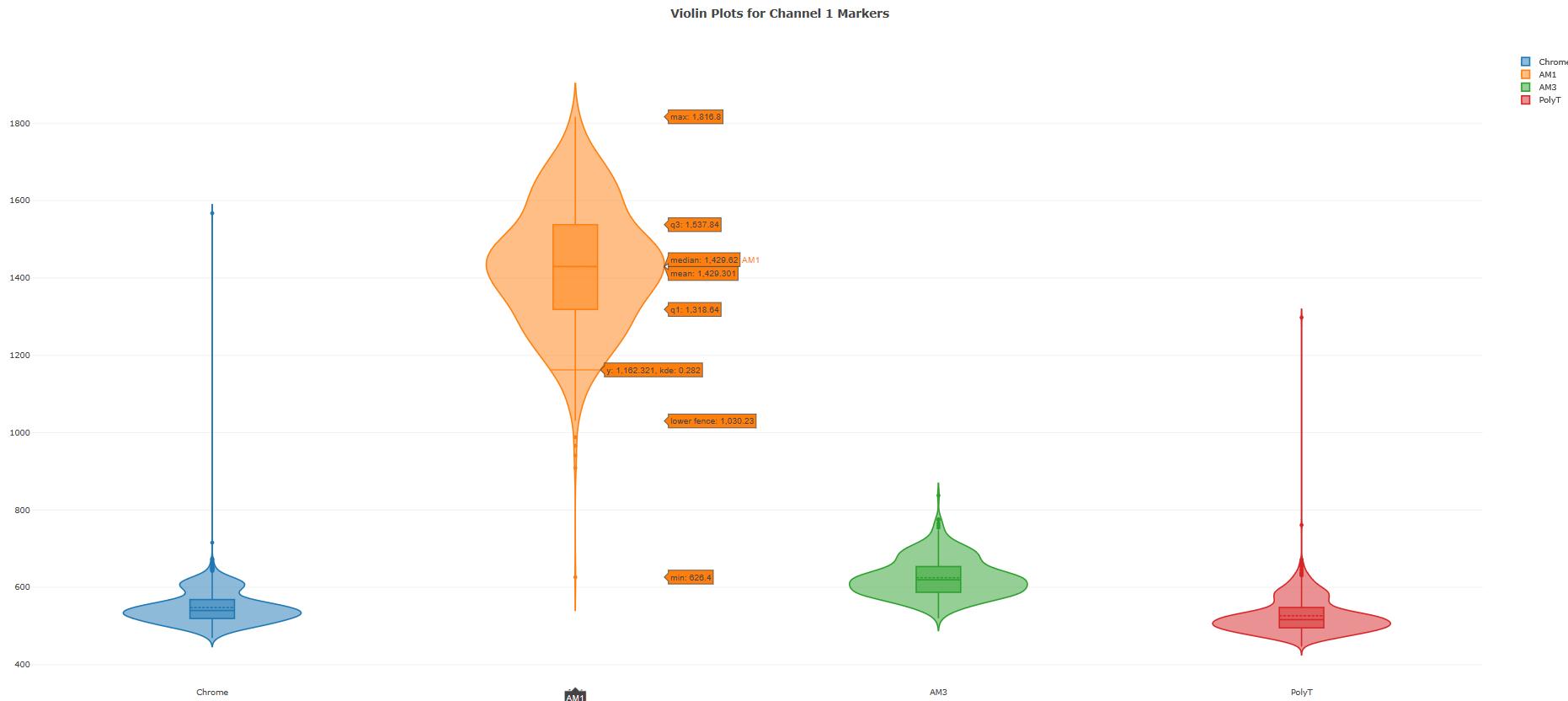
NP call: 94.2308%

No Call Rate: 66.8298%

Misclassified data



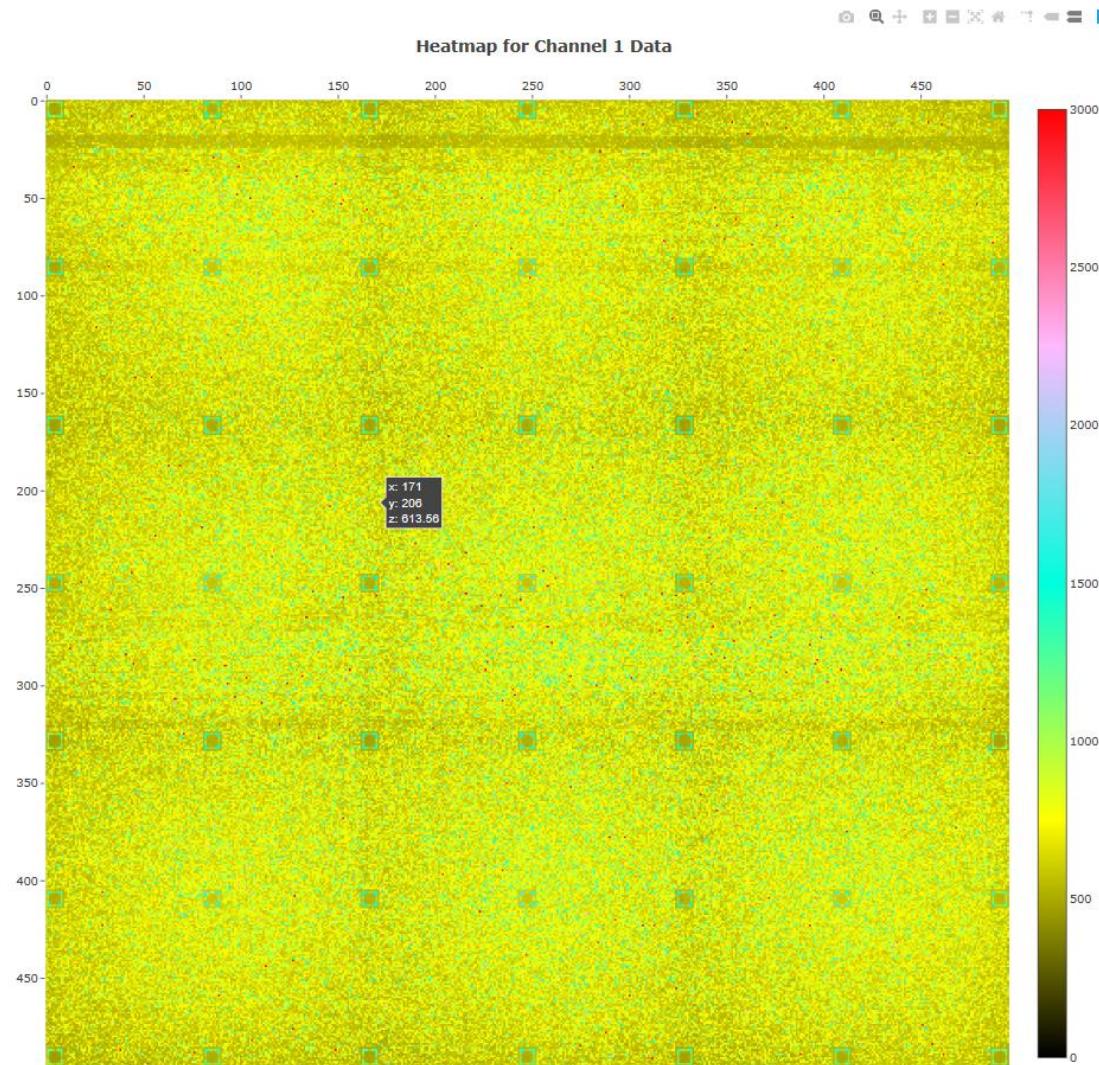
# Banff chip QC – Violin Plot



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# Banff chip QC – Heatmap



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# Banff chip QC – Marker Raw Data



marker\_ch1.csv - Excel

檔案 常用 插入 頁面配置 公式 資料 校閱 檢視 說明 搜尋

自動儲存 (○關閉) | 貼上 | 新細明體 | 12 | A<sup>+</sup> A<sup>-</sup> | 自動換行 | 通用格式 | \$ % , | 00 00 | 設定格式化 | 格式化為儲存格的條件 | 表格 | 樣式 | 插入 | 刪除 | 格式 | 儲存格 | 編輯 | 共用 | 註解 | 奇軒 何 | 回 | 一 | □ | × |

剪貼簿 | 字型 | 對齊方式 | 數值 | 樣式 | 儲存格 | 編輯 |

A1 : fx 487.24

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	487.24	488.16	485.8	492.36	502.04	482.16	503.44	491.17	505.72	500.8	nan								
2	492.84	908.88	1176.56	1107.6	604.4	610.56	1087.88	1232.97	1247.84	512.56	nan								
3	505.84	941	503.44	487.92	471.76	481.48	501.96	520.93	1232.72	521.52	nan								
4	512.08	626.4	499.52	493.76	471.12	500.76	500.08	515.4	1231.2	503.76	nan								
5	496.16	561.28	493.92	488.36	489.32	492.52	486.2	503.97	542.04	496.28	nan								
6	501.4	569.76	504.92	534.2	480.52	498.84	485.92	516.13	621.84	524.84	nan								
7	499.88	1113.76	501.52	489.4	500.6	477.44	506.6	490.5	1230.76	521	nan								
8	514.33	1297.9	525.1	479.67	493.3	478.57	508.27	502.22	1192.9	547.2	nan								
9	520.96	1349.44	1247.16	1273.52	683.08	607	1319.56	1300.5	1266.76	526.32	nan								
10	502.52	514.36	528.24	507.64	513.48	503.16	524.92	520.63	537.84	509.72	nan								
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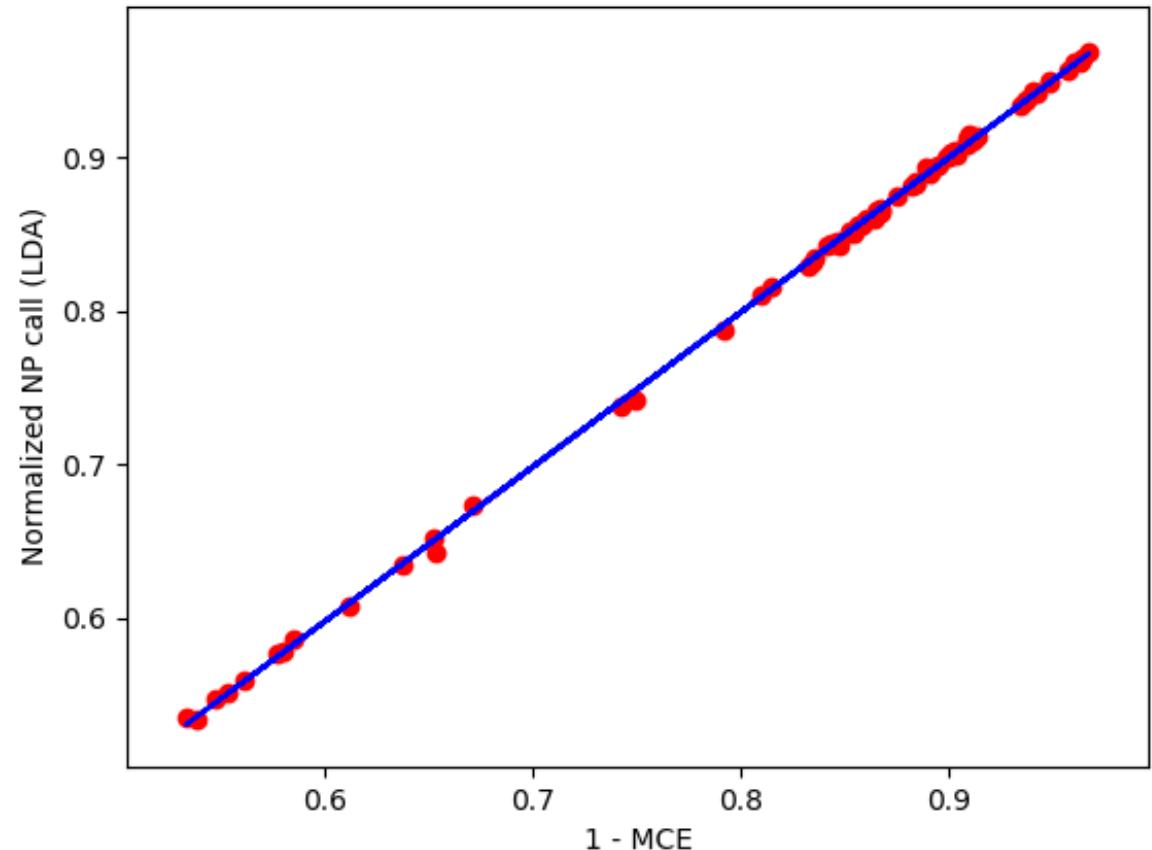
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# Correlation and Regression analysis (MCE vs. NP call)



MCE vs. NPcall (Linear Regression)



Training data: 80% data

Model:  $NPcall = -0.005 + 1.005 \cdot (1 - MCE)$   
 $R^2: 99.96\%$

Testing data: 20% data

MSE: 5.999574703240224e-06

It still need NP data to calculate the MCE.