Semester's Thesis Defense

Image Processing & Nanoparticle Quantification for Effective Biomarker Detection in Sepsis Diagnostics

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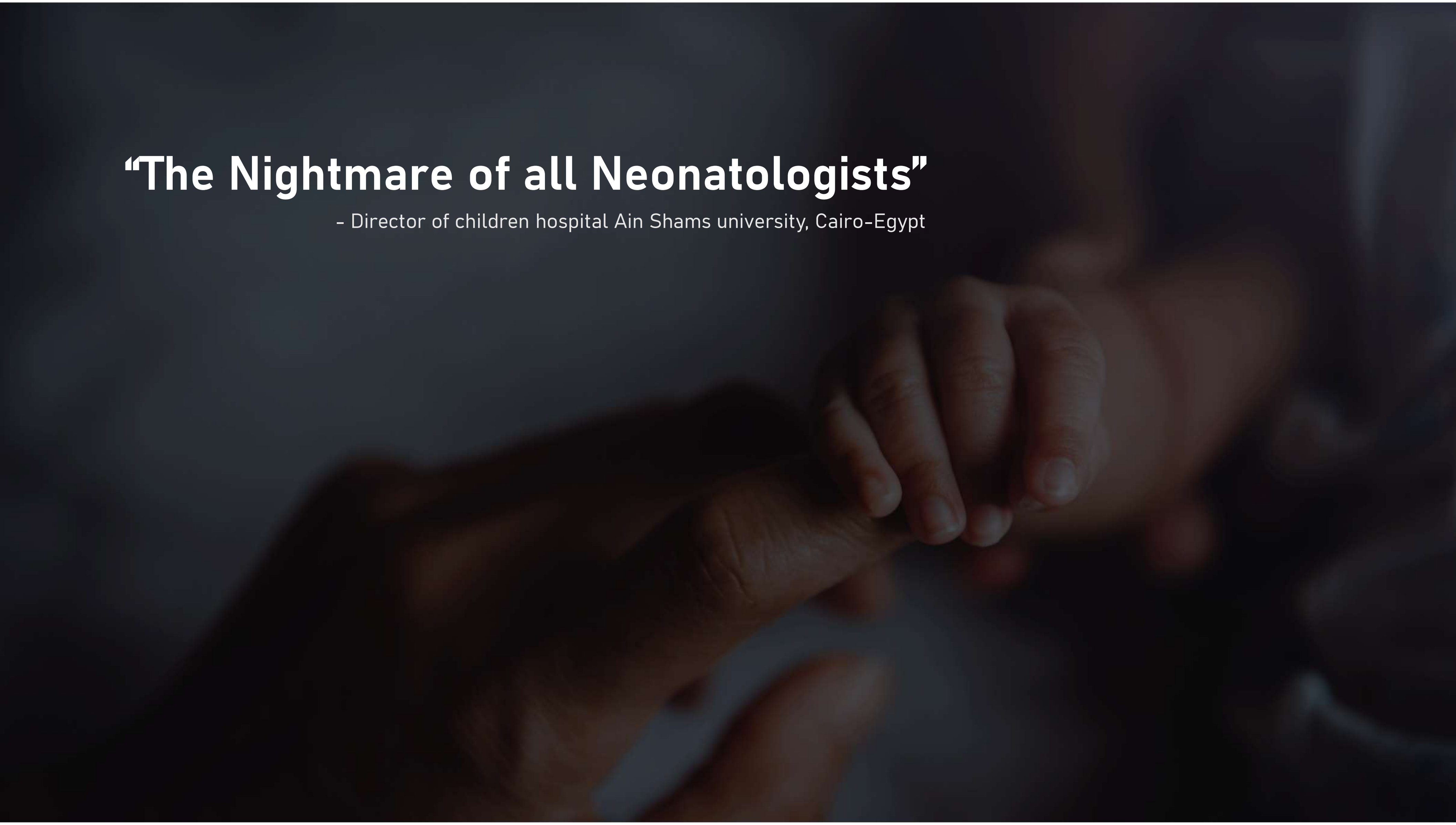
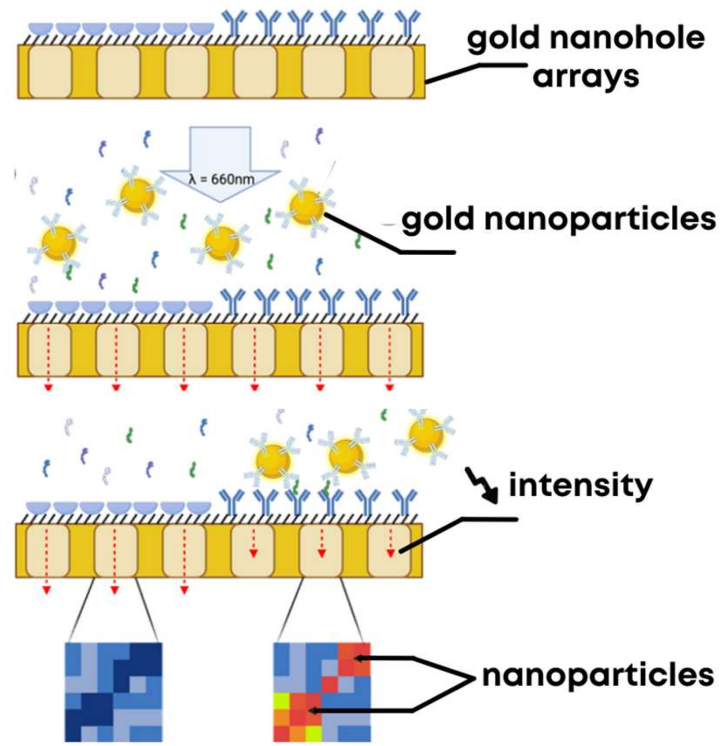
08/01/2024


"The Nightmare of all Neonatologists"
- Director of children hospital Ain Shams university, Cairo-Egypt

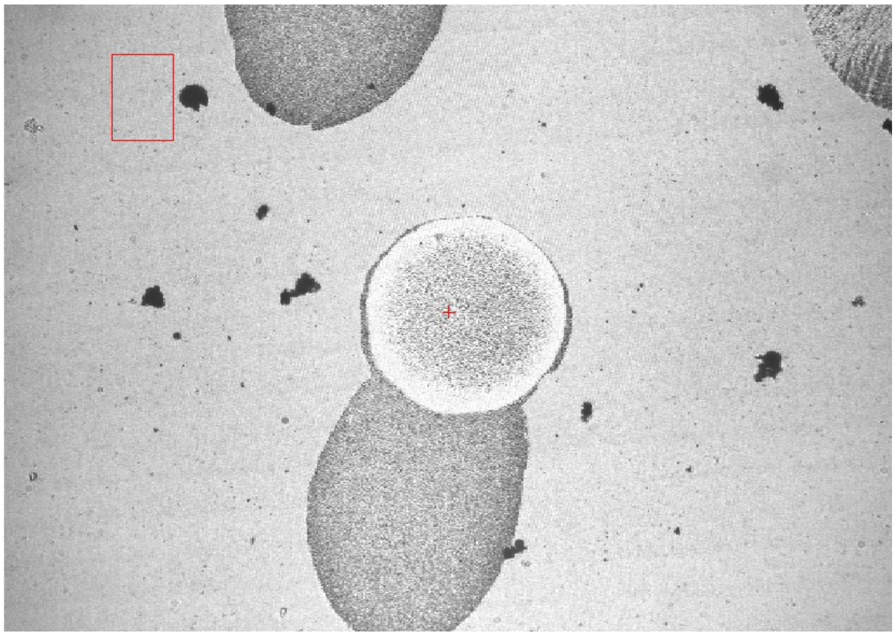
Neosens, a Start-up in Creation

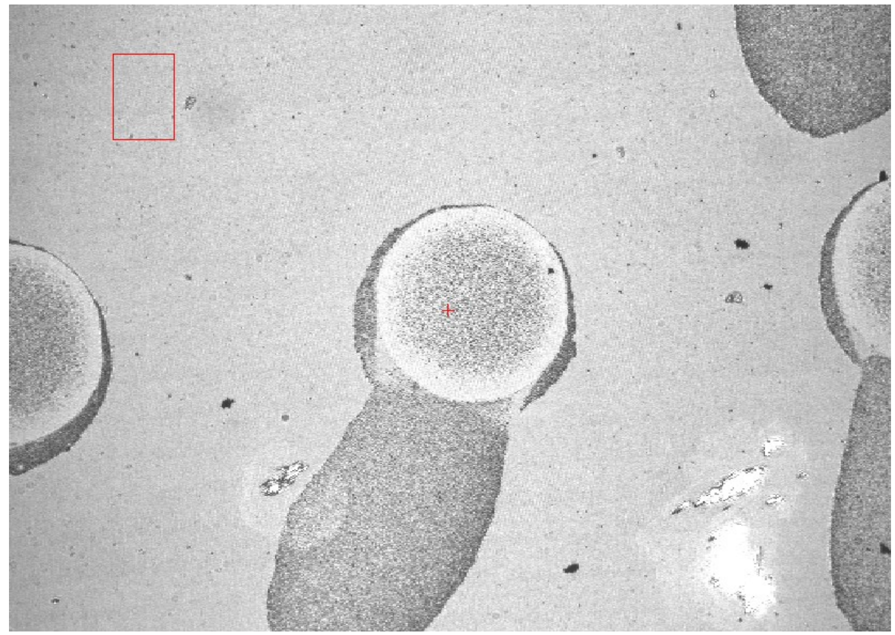
A Promising Biosensing Technology

- Plasmonic Biosensor
- Extraordinary Optical Transmission
- Intensity Heatmap



Find an Optimal Pipeline





- Count Individual Gold Nanoparticle (AuNP)
- Achieve a LOD < 10pg/mL
- In a 5min time-frame

Literature Research: Endpoint Analysis

Reduce background noise

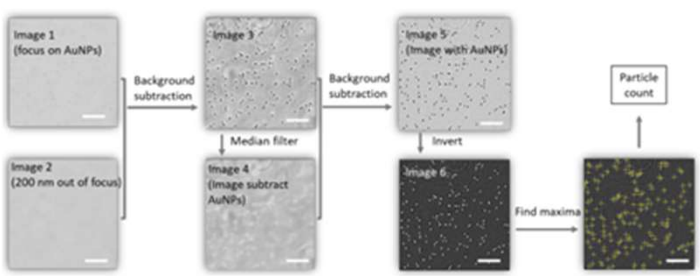
Filtering:

- Local Averaging Methods

$$I(x,y) * m(x,y) = \sum_{i=-\frac{w}{2}}^{\frac{w}{2}} \sum_{j=-\frac{w}{2}}^{\frac{w}{2}} I(x+i, y+j)m(i,j)$$

- K-space filtering

Background subtraction [1]:



Increase contrast

CLAHE

AuNPs identification

Global thresholding algorithm:

- Global fixed thresholding
- Global median thresholding

Local adaptive thresholding algorithm

[1]: W. Jing, Y. Wang, C. Chen, F. Zhang, Y. Yang, G. Ma, E. H. Yang, C. L. N. Snozek, N. Tao, and S. Wang, "Gradient-based rapid digital immunoassay for high-sensitivity cardiac troponin t (hs-ctnt) detection in 1 l plasma," ACS Sensors, vol. 6, no. 2, pp. 399–407, 2021, pMID: 32985183.

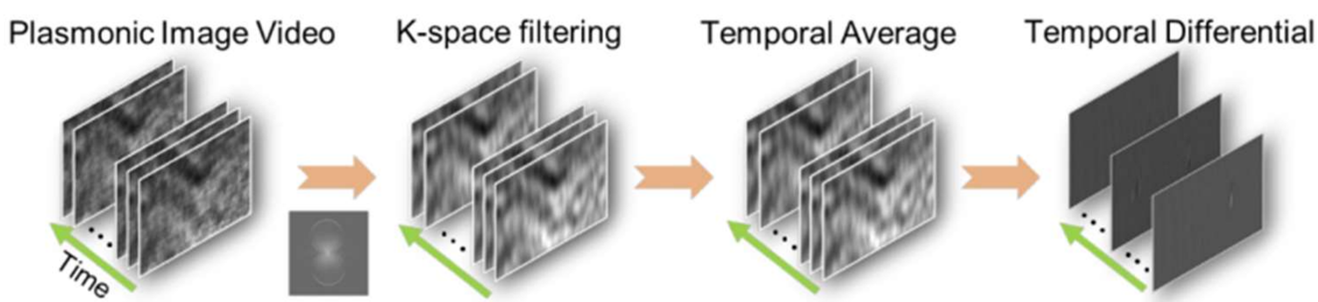
Literature Research: Time-resolved Analysis

Reduce background noise

AuNPs identification

Temporal Average $$\bar{I} = \frac{1}{n}\sum_{i=1}^{n} I_i$$

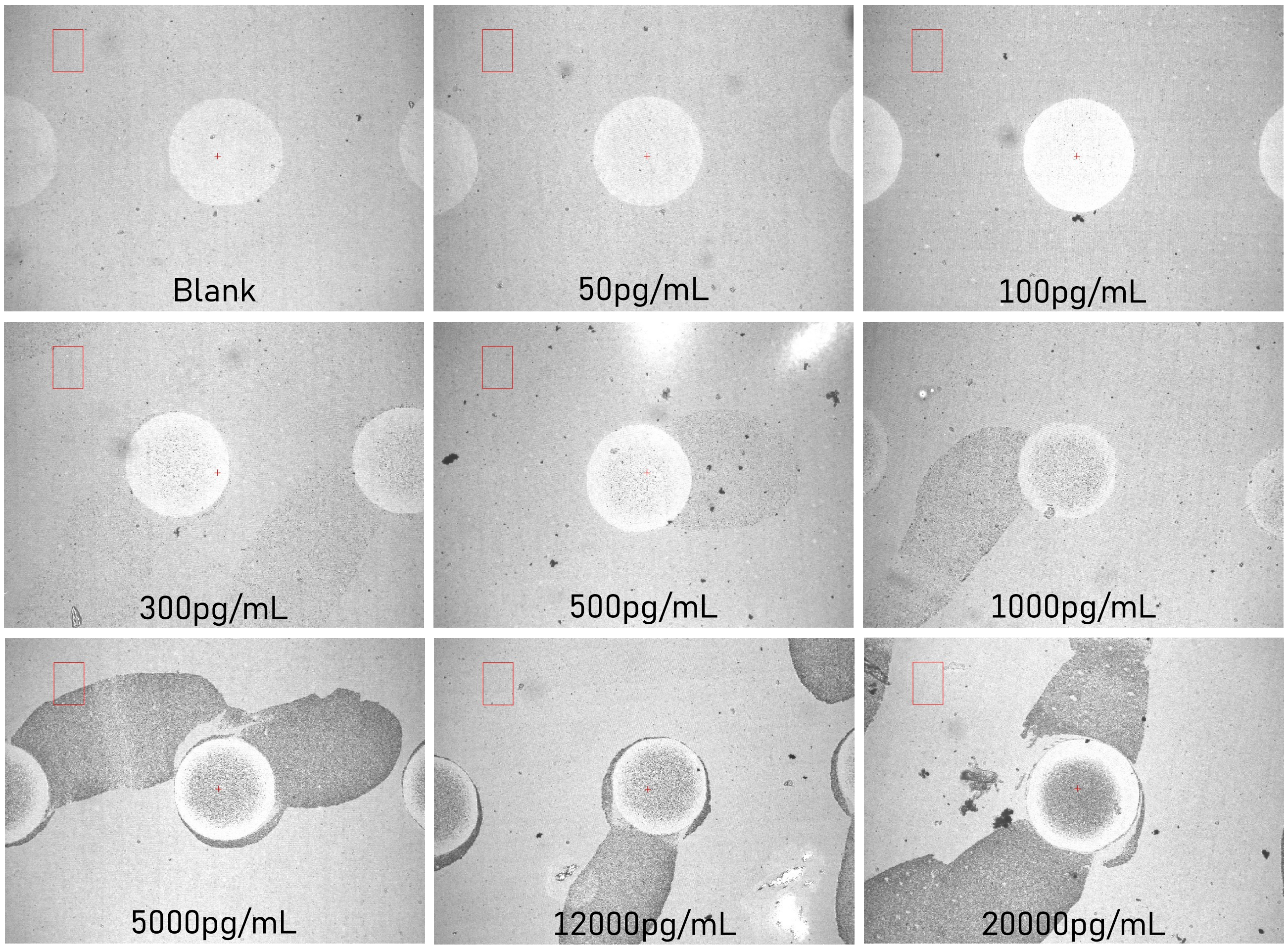
Differential Analysis



[2]

[2]: W. Jing, Y. Wang, Y. Yang, Y. Wang, G. Ma, S. Wang, and N. Tao, "Time-resolved digital immunoassay for rapid and sensitive quantitation of procalcitonin with plasmonic imaging," ACS Nano, vol. 13, no. 8, pp. 8609–8617, 2019, pMID: 31276361.

Our Endpoint Database



Methods & Discussion

Unit Testing: AuNPs Identification

CVL Computer Vision Lab

Metric Used

- Averaged pixel count per concentration
- Standard deviation of pixel count per concentration
- Limit of Detection (LoD)

Global Fixed Thresholding Algorithm

- Iteration over 40 threshold values ∈ [0,255]

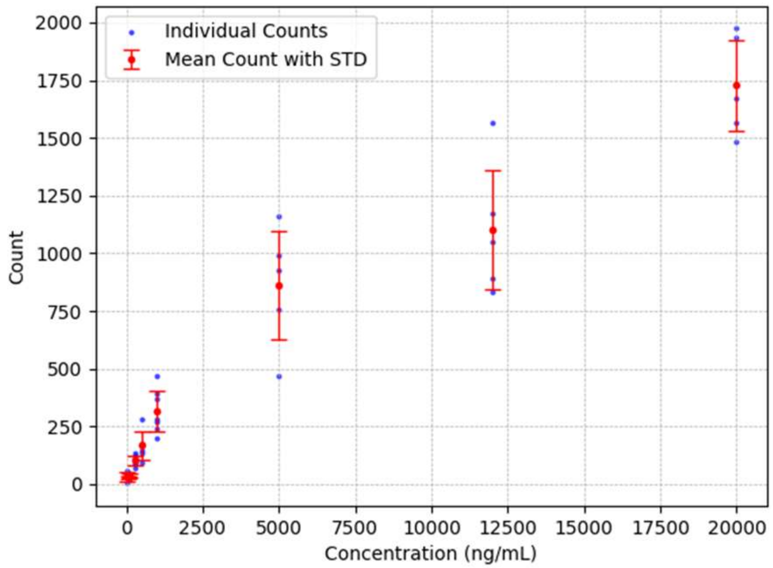
Table 4.1: Examples of average white pixel counts for different threshold values

Concentration (ng/mL)	Threshold Value				
	100	115	135	155	180
0	23	24	32	43	82
0.05	23	24	30	45	82
0.1	24	27	36	52	82
0.3	25	45	102	215	491
0.5	68	92	168	321	845
1	32	103	317	782	2067
5	128	328	860	1793	3801
12	55	312	1102	2304	4474
20	39	274	1727	4429	8556

Table 4.2: Examples of standard deviation of white pixel counts for different threshold values

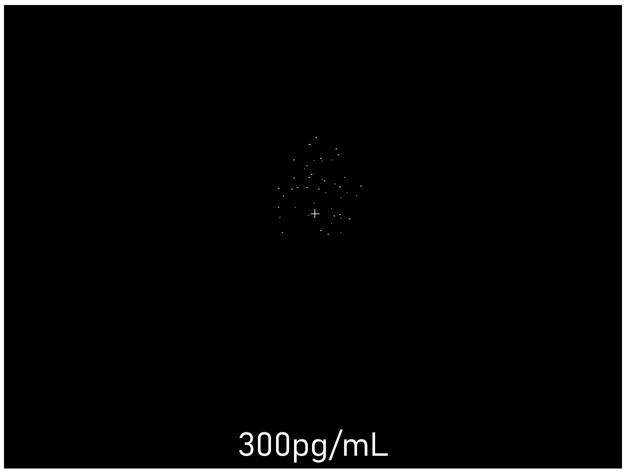
Concentration (ng/mL)	Threshold Value				
	100	115	135	155	180
0	18	18	23	24	34
0.05	0	1	6	10	21
0.1	1	4	9	11	13
0.3	3	9	25	52	90
0.5	58	62	70	113	697
1	28	61	95	117	192
5	75	134	261	383	580
12	32	139	291	448	687
20	25	212	219	987	1860

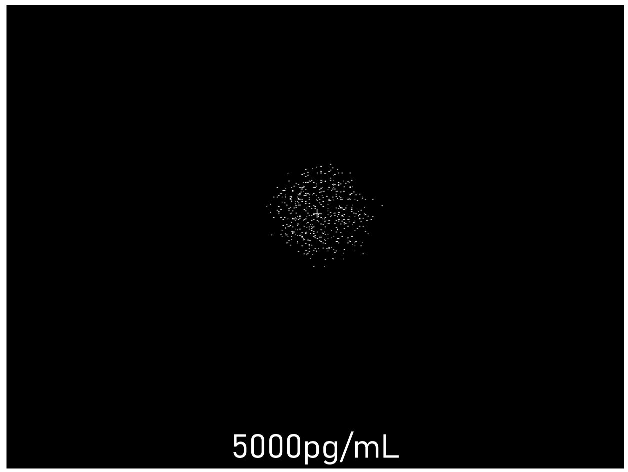
Optimal threshold = 135

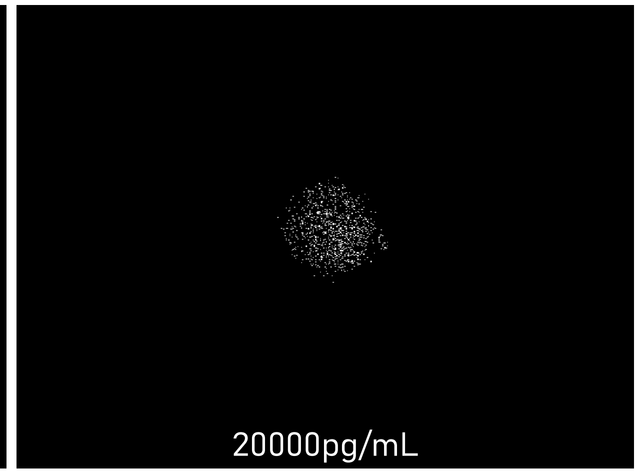


LoD ~ 50pg/mL

Global Fixed Thresholding Algorithm







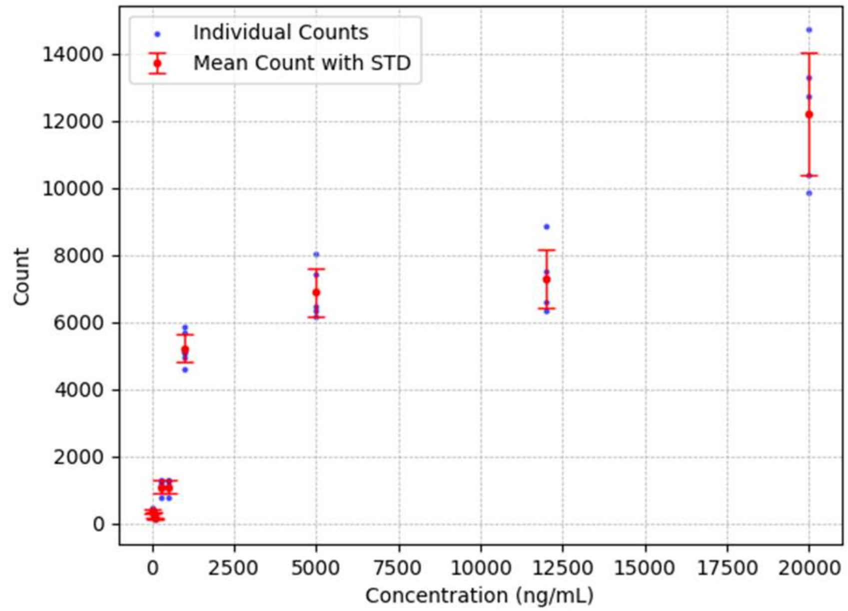
Global Median Thresholding Algorithm

Threshold value

=

Median value of blank samples [5]

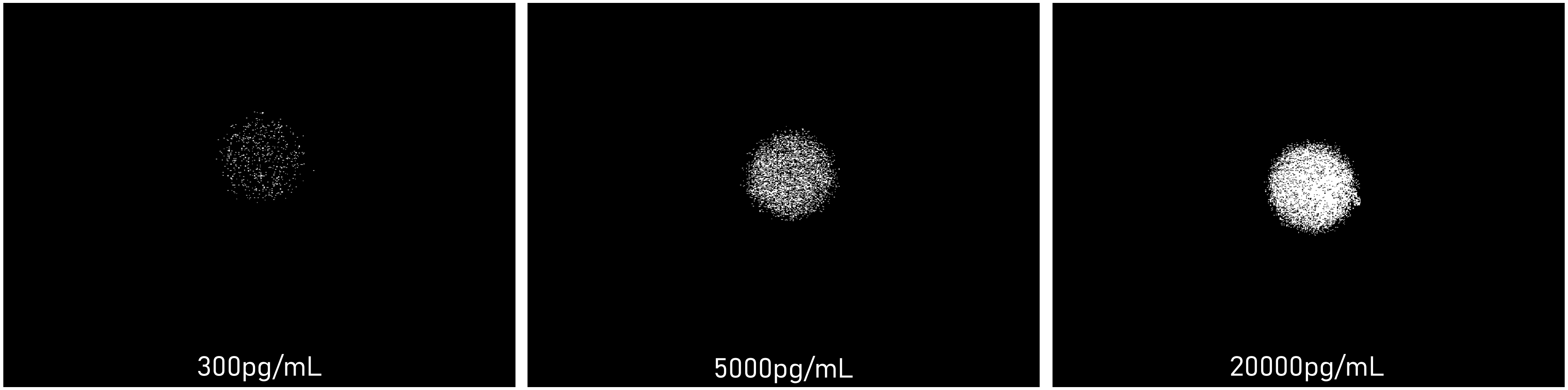
Calculated threshold = 203



Poor **LoD** & high number of **false positive**

[5]: W. Zhang, T. Dang, Y. Li, J. Liang, H. Xu, G. L. Liu, and W. Hu, "Digital plasmonic immunosorbent assay for dynamic imaging detection of protein binding," Sensors and Actuators B: Chemical, vol. 348, p. 130711, 2021.

Global Median Thresholding Algorithm



Local Adaptive Thresholding Algorithm

- Iteration over 120 combinations with window size ∈ [3,21] & Constant ∈ [0,50]

Fixed window size

Table A.1: Examples of average white pixel counts for different adaptive constant values (window size = 3)

Concentration (ng/mL)	Constant Value				
	2	10	15	25	40
0	9727	3531	1482	204	44
0.05	9015	3502	1635	315	90
0.1	6796	2557	1246	283	103
0.3	8146	4325	2804	1266	565
0.5	8613	4234	2611	1137	494
1	9874	6401	4727	2578	1099
5	9649	6914	5557	3706	2004
12	9469	7037	5883	4175	2424
20	10385	7550	6017	3590	1440

Table A.2: Examples of standard deviation of white pixel counts for different adaptive constant values (window size = 3)

Concentration (ng/mL)	Constant Value				
	2	10	15	25	40
0	49	207	140	44	19
0.05	674	572	301	57	11
0.1	997	609	335	81	18
0.3	778	498	301	168	83
0.5	909	625	447	183	74
1	144	194	213	220	137
5	311	244	199	156	97
12	414	361	304	209	100
20	456	174	368	731	770

Fixed constant

Table A.3: Examples of average white pixel counts for different adaptive window sizes (Constant = 15)

Concentration (ng/mL)	Window Size				
	3	5	11	15	19
0	1482	2093	2148	2166	2209
0.05	1635	2147	2241	2282	2306
0.1	1246	1639	1720	1728	1744
0.3	2804	3216	3238	3247	3288
0.5	2611	3014	3074	3080	3075
1	4727	5134	5150	5172	5159
5	5557	5873	5911	5904	5893
12	5883	6150	6221	6241	6284
20	6017	6454	6656	6728	6766

Table A.4: Examples of standard deviation of white pixel counts for different adaptive window sizes (Constant = 15)

Concentration (ng/mL)	Window Size				
	3	5	11	15	19
0	140	308	229	223	244
0.05	301	383	353	361	358
0.1	335	432	413	408	408
0.3	301	403	339	347	355
0.5	447	444	451	452	466
1	213	176	154	141	143
5	199	208	204	215	215
12	304	300	309	321	322
20	368	285	266	209	193

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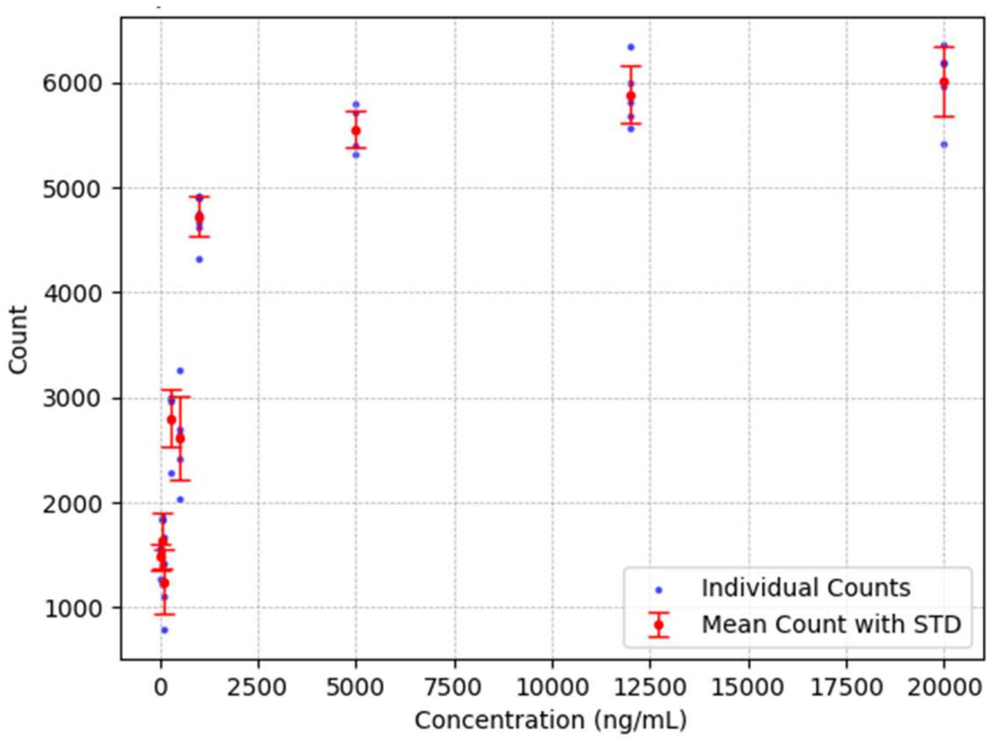
Local Adaptive Thresholding Algorithm

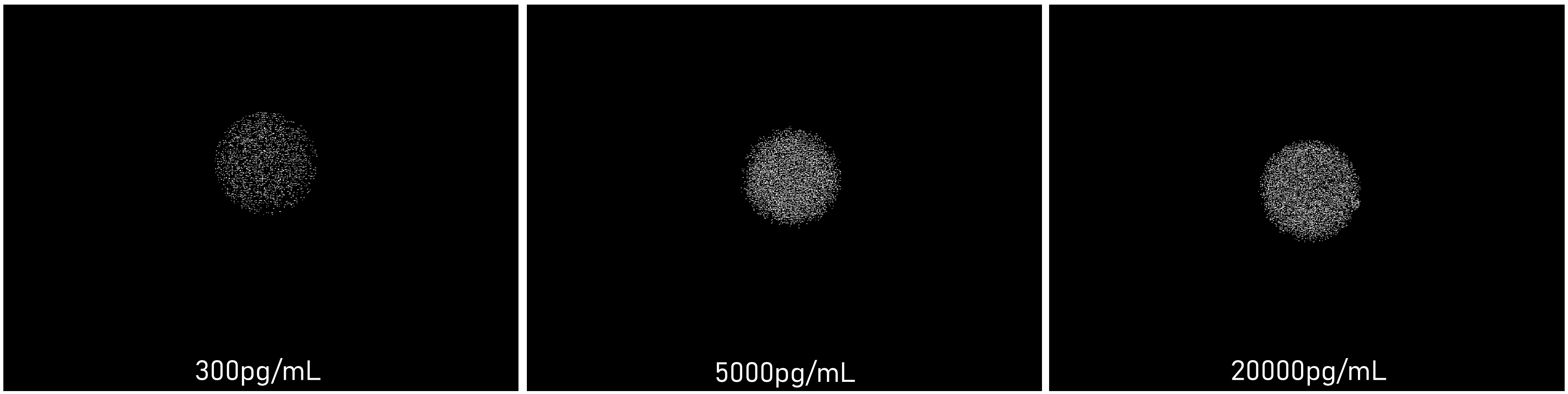
Optimal combination: window size = 3 – constant = 15



LoD ~ 100pg/mL

Large Standard Deviation

Local Adaptive Thresholding Algorithm



Methods & Discussion

Unit Testing: Noise Reduction

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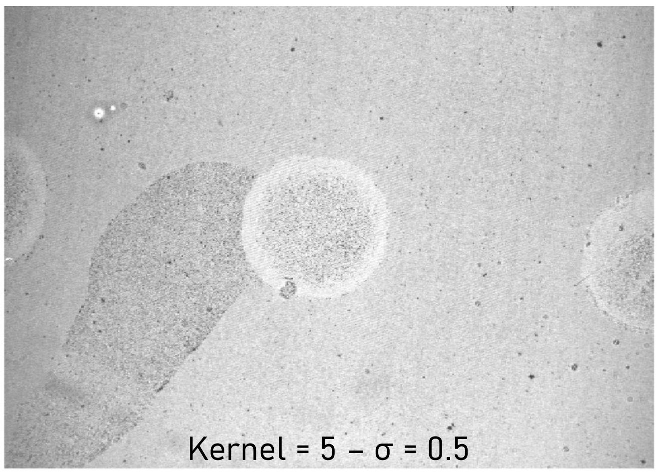
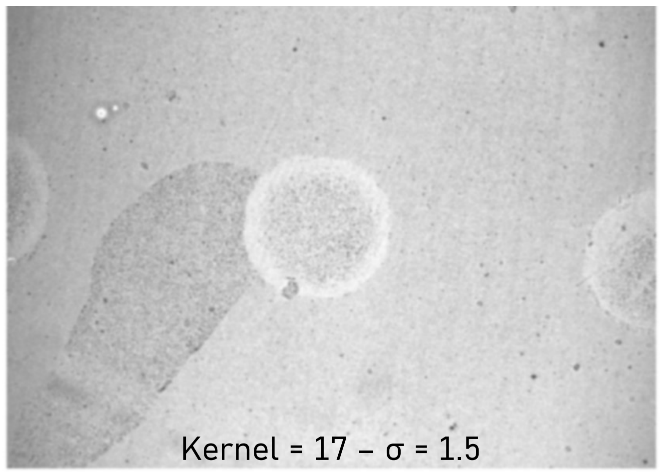
Metric Used

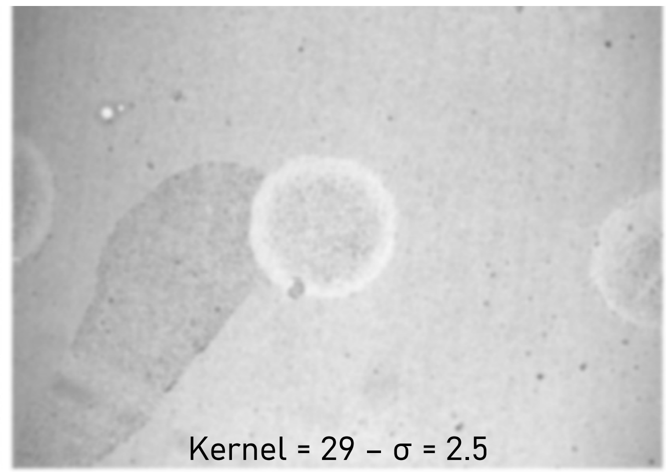
- Signal-to-noise ratio (SNR)
- Contrast-to-noise ratio (CNR)
- Weber contrast
- Structural similarity index measure (SSIM)

Gaussian Filtering

- Iteration over 25 combinations with Kernel size ∈ [5,29] & Sigma ∈ [0.5,2.5]







Gaussian Filtering

- Iteration over 25 combinations with Kernel size ∈ [5,29] & Sigma ∈ [0.5,2.5]

Table 4.4: Gaussian filtered images quality metrics

Filter		SNR	CNR	Weber Contrast	SSIM
Raw		**2.38**	**1.07**	**0.558**	**1.00**
Kernel	**Sigma**				
5	0.5	2.77	0.70	0.346	0.35
5	1.0	2.74	0.64	0.294	0.34
5	1.5	2.82	0.53	0.274	0.33
5	2.0	2.58	0.41	0.212	0.32
5	2.5	2.69	0.45	0.204	0.32
11	0.5	2.61	0.39	0.200	0.35
11	1.0	2.51	0.24	0.104	0.34
11	1.5	2.27	0.12	0.054	0.33
11	2.0	2.18	0.12	0.067	0.32
11	2.5	2.43	0.11	0.063	0.32
17	0.5	2.30	0.13	0.063	0.35
17	1.0	2.31	0.10	0.048	0.34
17	1.5	2.34	0.10	0.054	0.33
17	2.0	2.34	0.14	0.046	0.32
17	2.5	2.52	0.19	0.088	0.32
23	0.5	2.39	0.09	0.045	0.35
23	1.0	2.38	0.09	0.043	0.34
23	1.5	2.36	0.13	0.067	0.33
23	2.0	2.32	0.11	0.063	0.32
23	2.5	2.49	0.12	0.065	0.32
29	0.5	2.40	0.09	0.049	0.35
29	1.0	2.43	0.11	0.054	0.34
29	1.5	2.33	0.11	0.047	0.33
29	2.0	2.37	0.16	0.088	0.32
29	2.5	2.49	0.09	0.046	0.32

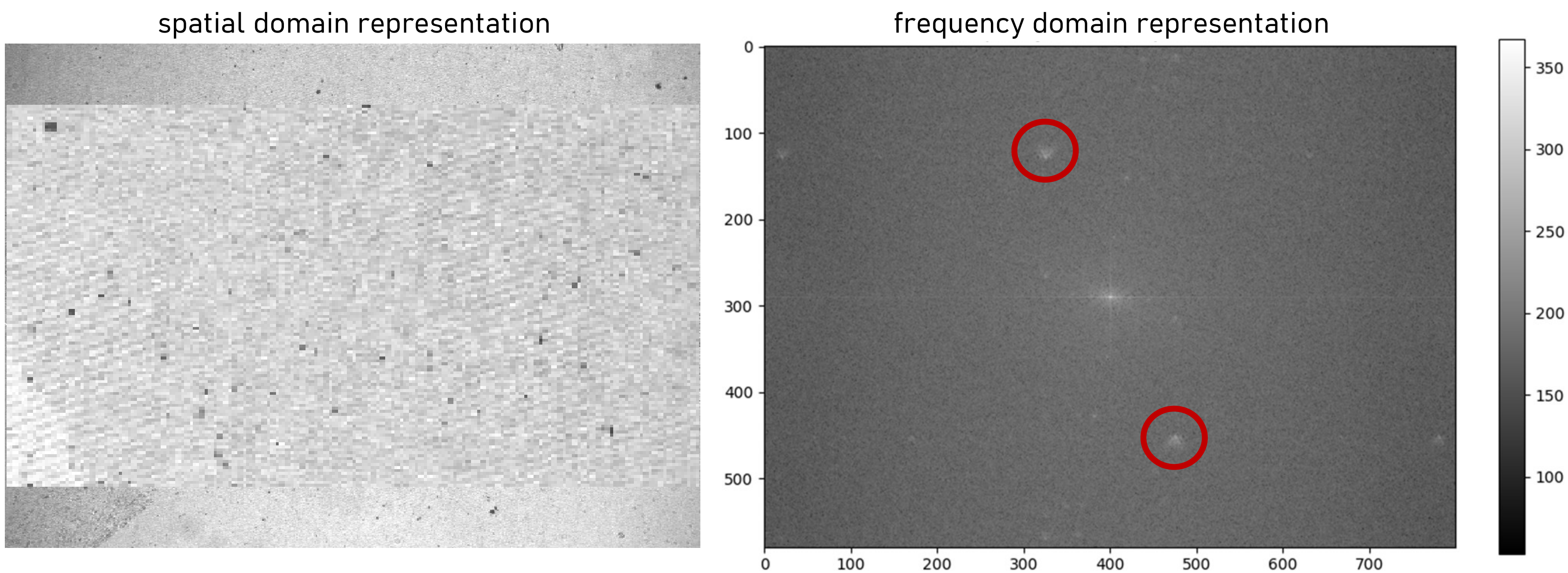
<u>Optimal combination</u>

Kernel = 5 – σ = 0.5

↓

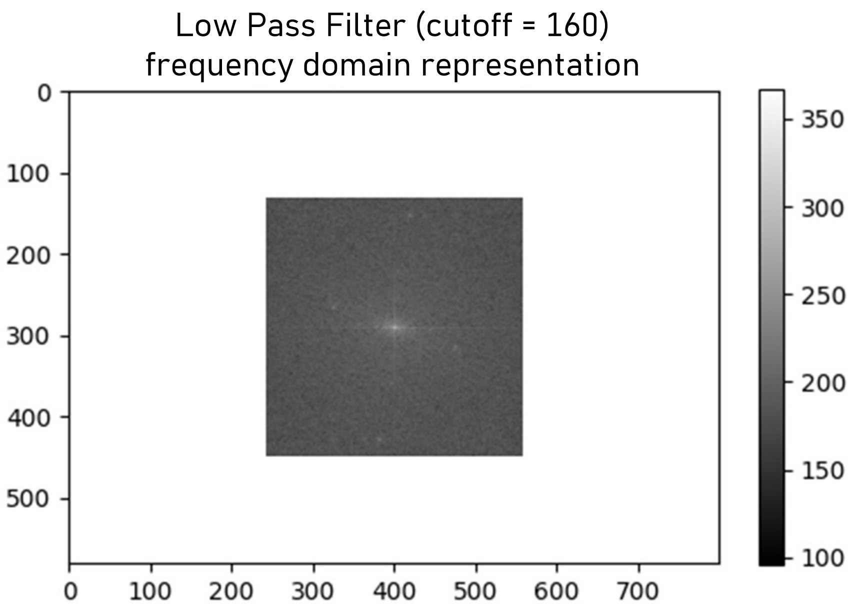
SNR increased by **16%**

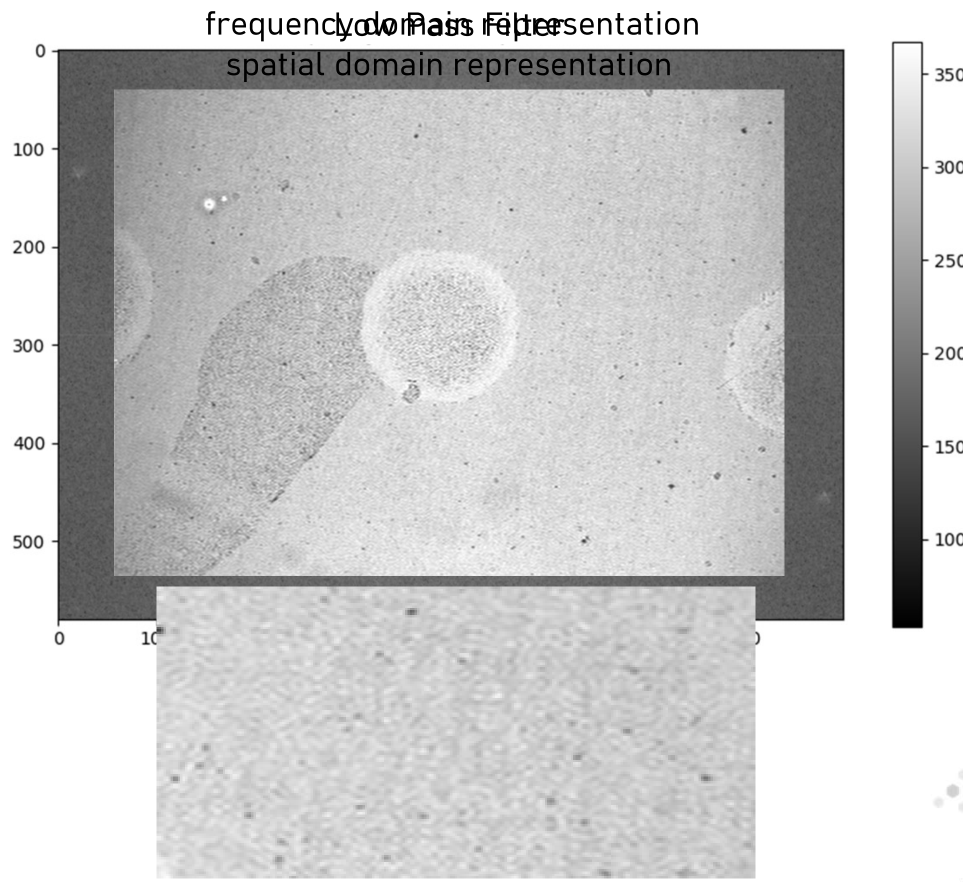
K-space filtering



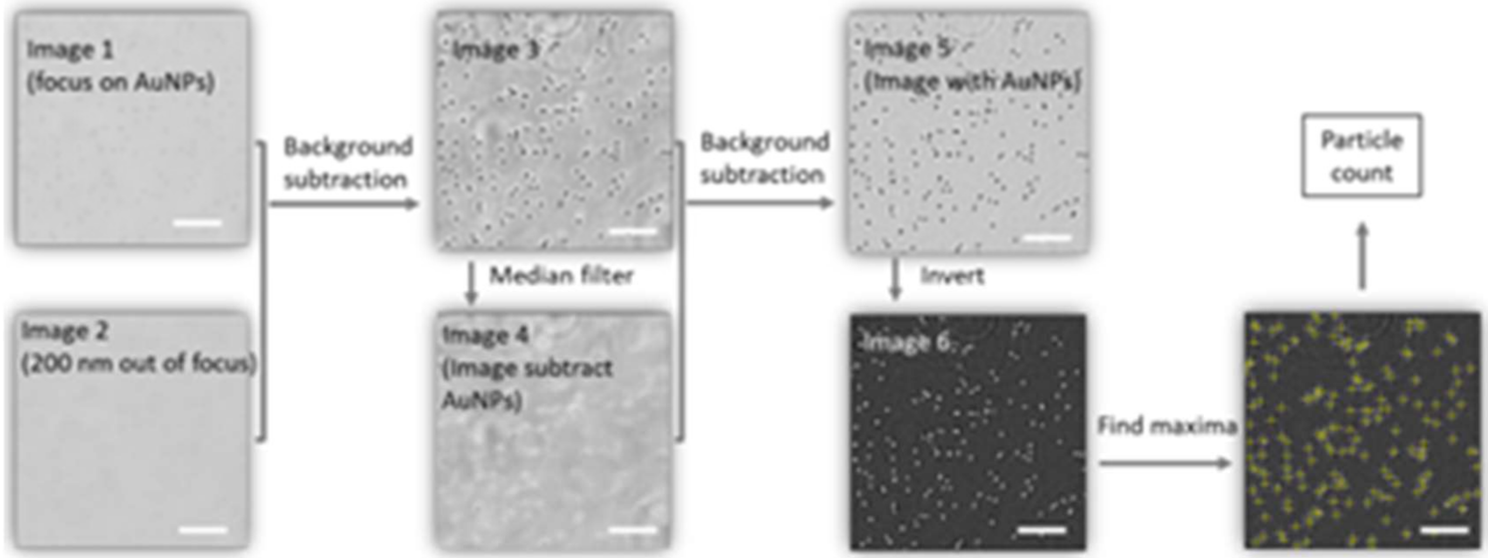
1. Instrumentation artifacts
2. Uniform brightness across the spectrum

K-space filtering





Background Subtraction

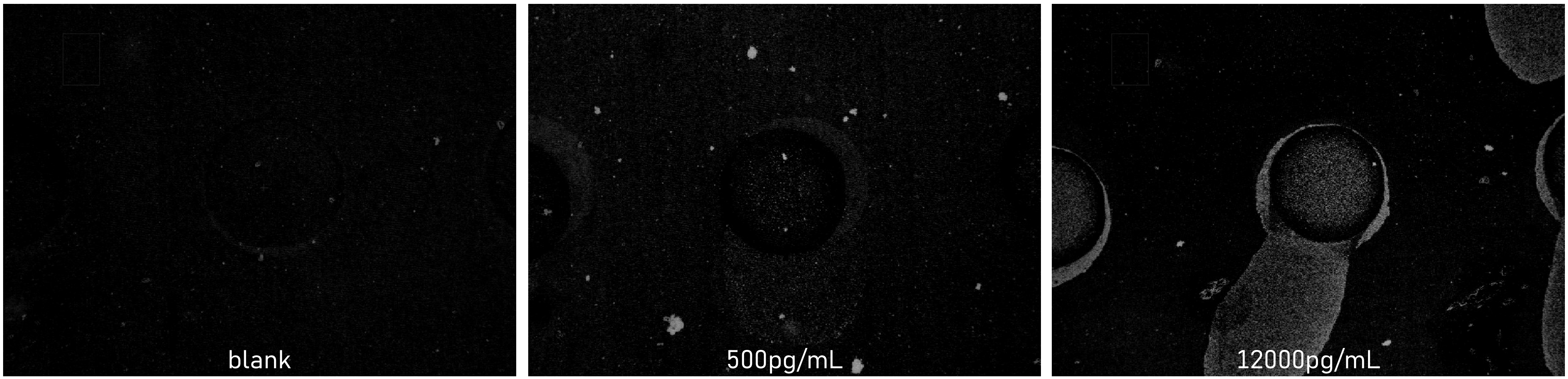


Step 1

Step 2

W. Jing, Y. Wang, C. Chen, F. Zhang, Y. Yang, G. Ma, E. H. Yang, C. L. N. Snozek, N. Tao, and S. Wang, "Gradient-based rapid digital immunoassay for high-sensitivity cardiac troponin t (hs-ctnt) detection in 1 l plasma," ACS Sensors, vol. 6, no. 2, pp. 399–407, 2021, pMID: 32985183.

Background Subtraction – Step 1



- Bound AuNPs
- Aggregated AuNPs
- Residual noise

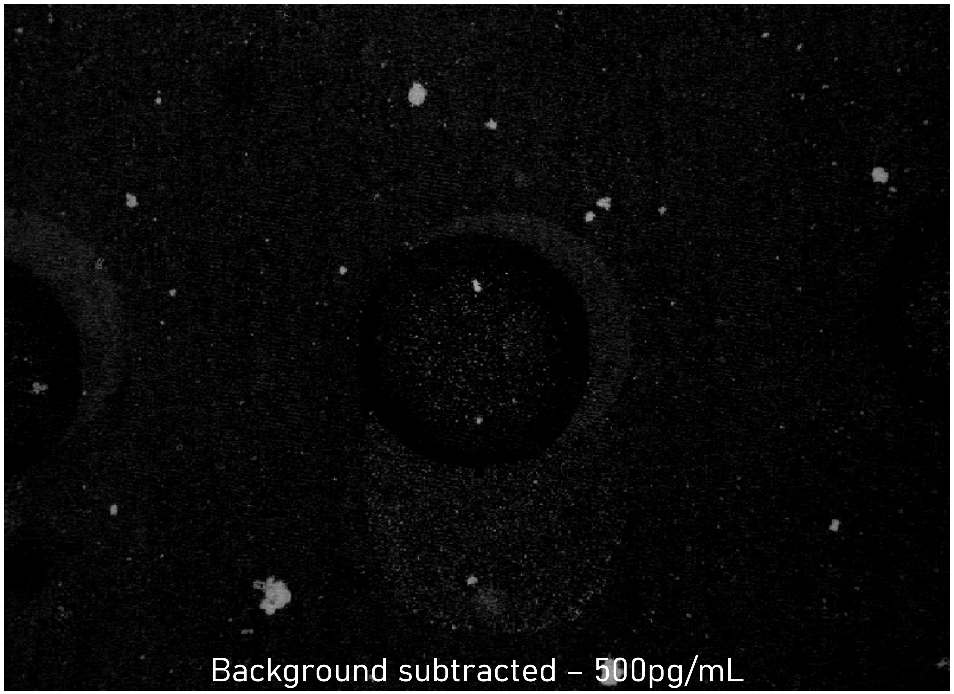
Background Subtraction – Step 1

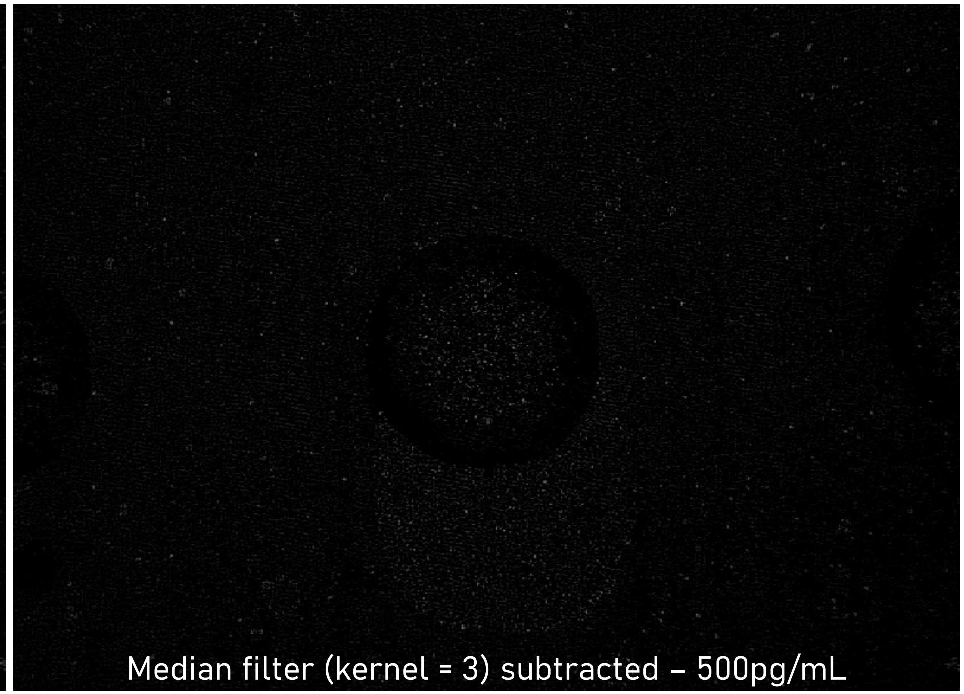
Table 4.5: Background subtracted images quality metrics

Concentration ($^{ng}/_{mL}$)	SNR	CNR	Weber Contrast	SSIM
0	0.6	0	0	0.01
0.05	0.5	0.13	0.21	0.01
0.1	0.2	0.44	0.696	0.01
0.3	0.7	0.07	0.114	0.01
0.5	0.8	0.19	0.301	0.01
1	2.7	2.06	3.268	0.01
5	3.5	2.82	4.484	0.01
12	4.3	3.64	5.79	0.01
20	6.7	6.06	9.624	-0.12
Average (Raw)	**2.38**	**1.07**	**0.56**	**1.00**
Average (Bg Subtracted)	**2.22**	**1.71**	**2.72**	**0.01**

- Poor background estimation
- Over-subtraction of the signal

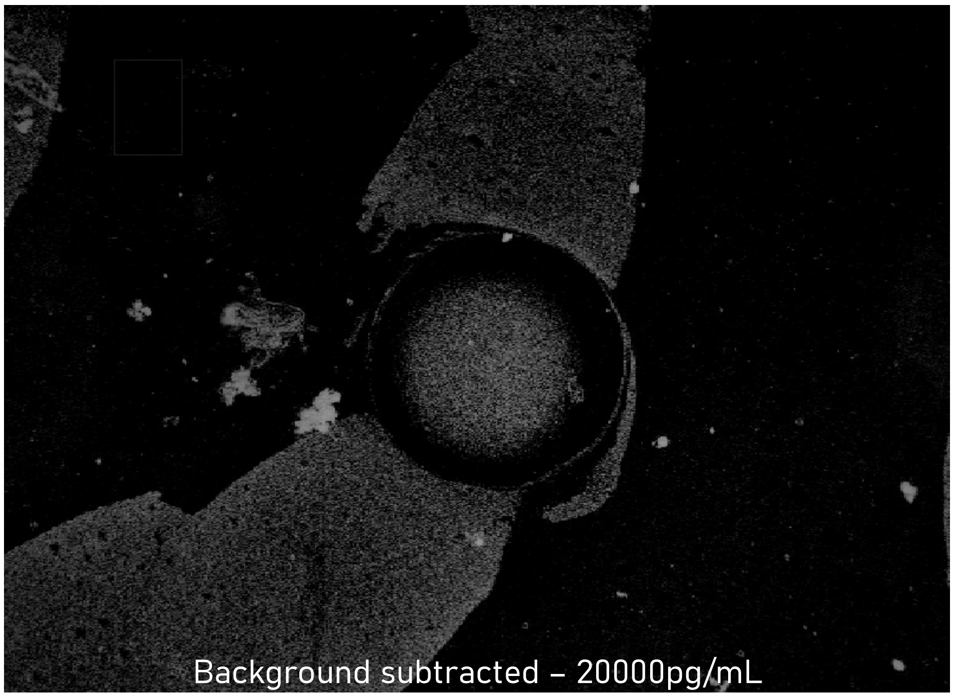
Background Subtraction – Step 2

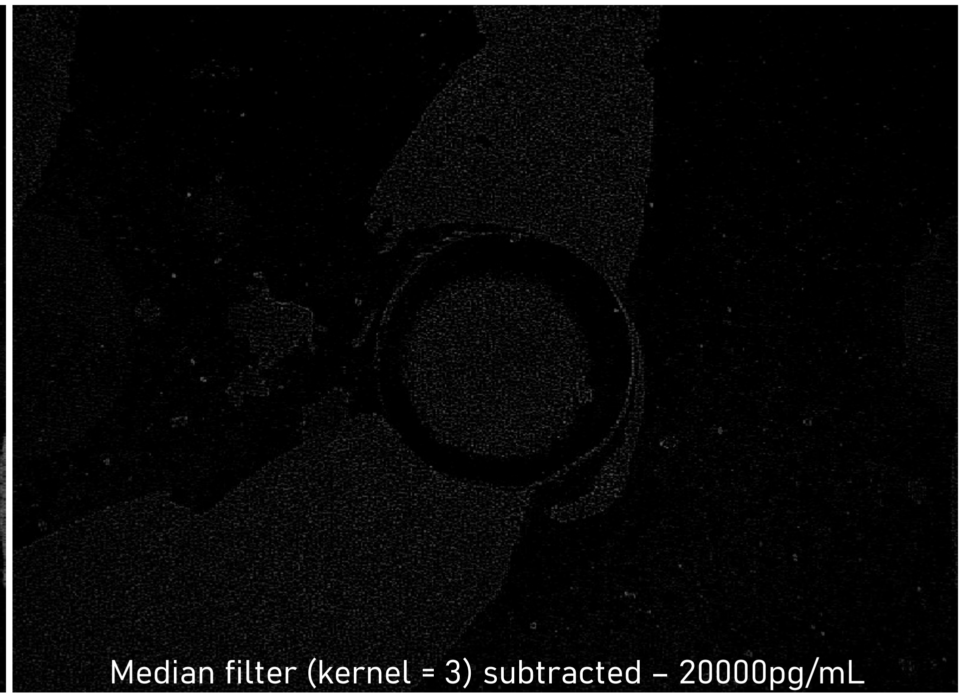




Suppression of aggregated AuNPs

Background Subtraction – Step 2





Important signal attenuation & high number of false negatives

Methods & Discussion

Integration Testing: Noise Reduction

Optimal Noise Reduction Pipeline

K-space filtering

cutoff frequency = 160

↓

Gaussian filtering

kernel = 5 – σ = 0.5

Optimal Noise Reduction Pipeline

Table 4.3: Raw Images Quality Metrics

Concentration ($^{ng}/_{mL}$)	SNR	CNR	Weber Contrast	SSIM
0	1.9	0.00	0.00	1.0
0.05	1.4	0.48	0.25	1.0
0.1	0.8	1.14	0.60	1.0
0.3	1.3	0.62	0.33	1.0
0.5	1.4	0.48	0.25	1.0
1	2.9	0.95	0.50	1.0
5	3.0	1.06	0.56	1.0
12	3.7	1.76	0.92	1.0
20	5.0	3.12	1.63	1.0

Table 4.6: Optimal Set Quality Metrics

Concentration ($^{ng}/_{mL}$)	SNR	CNR	Weber Contrast	SSIM
0	2.9	0.0	0.00	0.23
0.05	2.5	1.4	0.25	0.23
0.1	1.3	1.9	0.59	0.22
0.3	2.0	1.0	0.33	0.21
0.5	2.0	0.7	0.25	0.22
1	5.1	1.5	0.50	0.21
5	4.8	3.0	0.75	0.19
12	5.9	2.9	0.92	0.20
20	5.9	3.8	1.63	0.62

- SNR increased by 50%
- CNR increased by 69%
- Weber contrast remained unchanged

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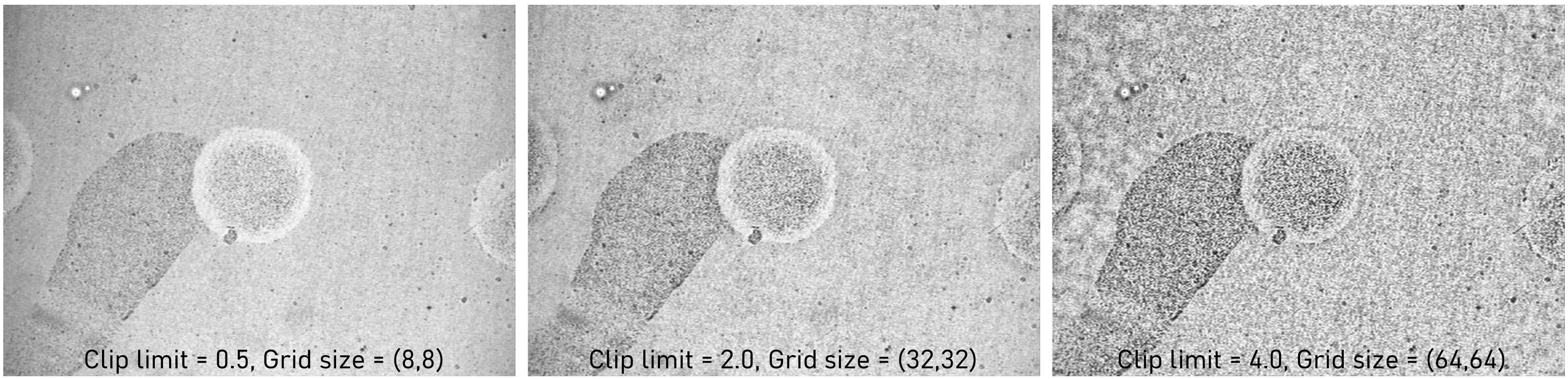
Methods & Discussion

Unit Testing: Contrast Enhancement

Contrast Enhancement - CLAHE

- Iteration over 20 combinations with Clip limit ∈ [0.5,8] & Grid size ∈ [(8,8),(64,64)]



Random noise persists and is amplified

Contrast Enhancement – CLAHE

- Iteration over 20 combinations with Clip limit ∈ [0.5,8] & Grid size ∈ [(8,8),(64,64)]

Table 4.7: Comparison of Quality Metrics

Set of Parameters		SNR	CNR	Weber Contrast	SSIM
Reference Image		**3.60**	**1.80**	**0.58**	**0.26**
Clip limit	**Grid size**				
0.5	(8,8)	3.58	1.74	0.58	0.39
0.5	(16,16)	3.58	1.73	0.57	0.40
0.5	(32,32)	3.54	1.76	0.57	0.41
0.5	(64,64)	3.07	1.60	0.60	0.27
1.0	(8,8)	3.45	1.62	0.61	0.34
1.0	(16,16)	3.37	1.80	0.55	0.34
1.0	(32,32)	3.29	1.76	0.58	0.41
1.0	(64,64)	3.07	1.60	0.60	0.27
2.0	(8,8)	3.46	1.75	0.61	0.25
2.0	(16,16)	3.32	1.78	0.55	0.25
2.0	(32,32)	3.31	1.75	0.59	0.29
2.0	(64,64)	3.07	1.60	0.61	0.27
4.0	(8,8)	3.51	1.82	0.61	0.15
4.0	(16,16)	3.25	1.94	0.59	0.15
4.0	(32,32)	3.07	1.87	0.63	0.16
4.0	(64,64)	3.02	1.76	0.68	0.15
8.0	(8,8)	3.21	1.85	0.64	0.09
8.0	(16,16)	3.04	2.07	0.67	0.08
8.0	(32,32)	2.97	1.80	0.64	0.08
8.0	(64,64)	2.85	1.66	0.61	0.08

- Maximum 17% Weber contrast increase

- Accompanied with a 18% SNR decrease

Final Pipeline

Final Pipeline for Endpoint Database

K-space filtering

cutoff frequency = 160

↓

Gaussian filtering

kernel = 5 – σ = 0.5

↓

ROI selection and masking

↓

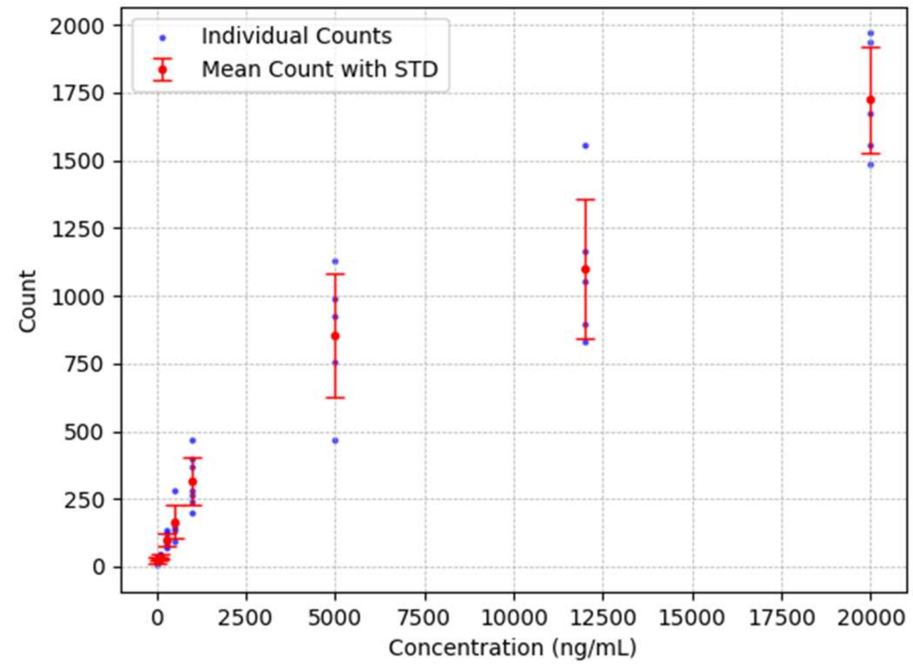
Global fixed thresholding algorithm

Threshold value = 135

Final Pipeline Results

Table 4.8: Results of our final pipeline

Concentration (ng/mL)	Mean	Standard Deviation
0	23	14
0.05	30	4
0.1	35	9
0.3	105	22
0.5	165	67
1	316	80
5	854	232
12	1099	254
20	1748	205



LoD < 50pg/mL

Time-Resolved Database

Unit Testing

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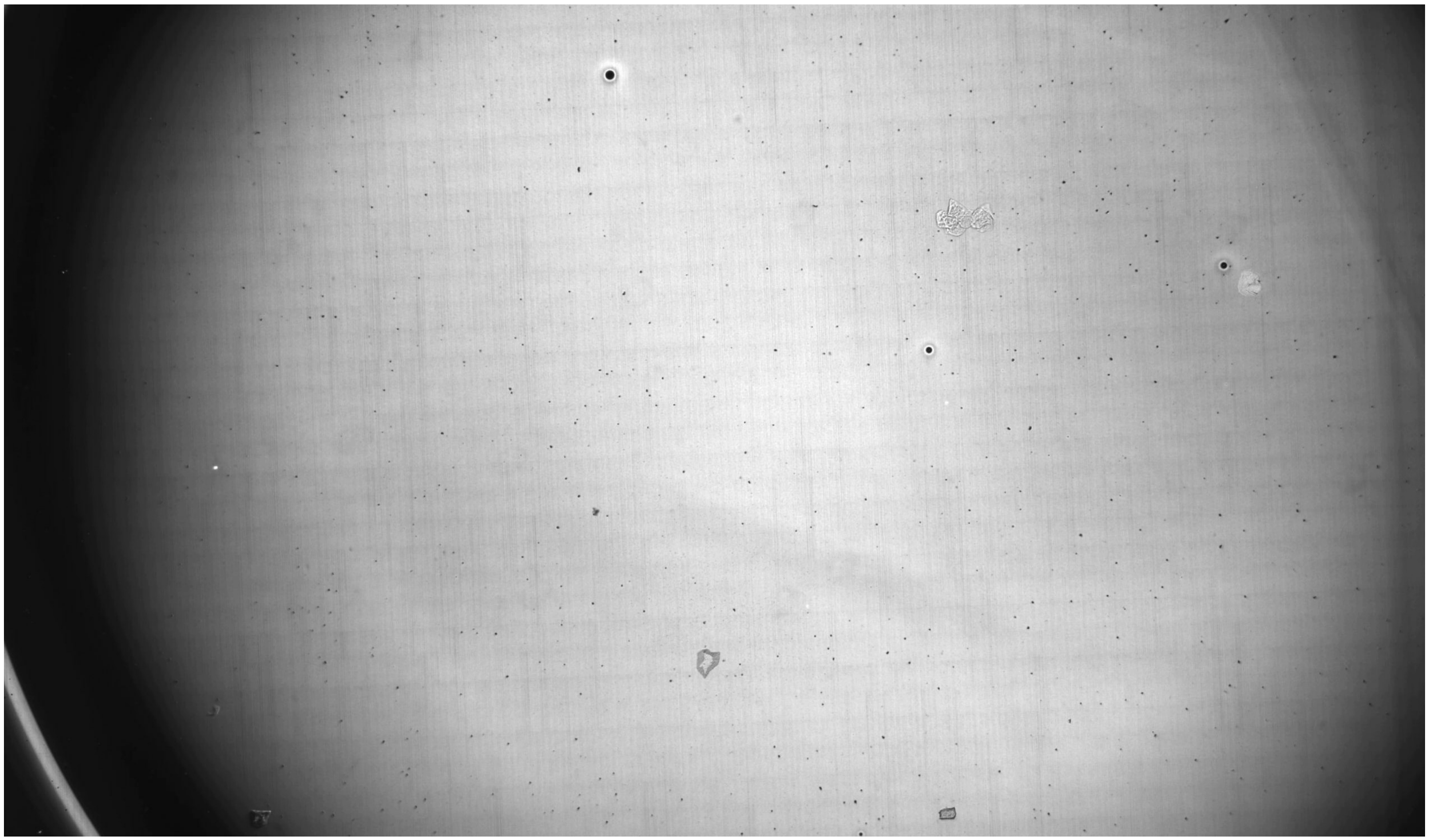
Creation of a New Database

- High-resolution video → image database
- 5 fps for 36min 30s → 10'900 images
- Low residual noise → not representative

Creation of a New Database



Temporal Average

- Computation of temporal average over 50 raw images

Table 5.1: Results of Temporal Averaging process

Frame	SNR	SSIM
frame 07051 - time: 23:30.00	6.7	1
frame 07052 - time: 23:30.20	6.7	1
frame 07053 - time: 23:30.40	6.7	1
frame 07054 - time: 23:30.60	6.7	1
frame 07055 - time: 23:30.80	6.7	1
frame 07056 - time: 23:31.00	6.7	1
frame 07057 - time: 23:31.20	6.7	1
frame 07058 - time: 23:31.40	6.7	1
...	...	...
frame 07093 - time: 23:38.40	6.8	1
frame 07094 - time: 23:38.60	6.8	1
frame 07095 - time: 23:38.80	6.8	1
frame 07096 - time: 23:39.00	6.8	1
frame 07097 - time: 23:39.20	6.8	1
frame 07098 - time: 23:39.40	6.8	1
frame 07099 - time: 23:39.60	6.8	1
frame 07100 - time: 23:39.80	6.8	1
averaged frame	**6.8**	**0.95**

- SNR remained unchanged
- Low random background noise
- Non-efficient against non-random noise

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CVL Computer Vision Lab

Feel free to ask any questions!

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