Application Note

[NOTE] How to Set Profile for Fluorescent Lateral Flow Test to Get the Best Performance

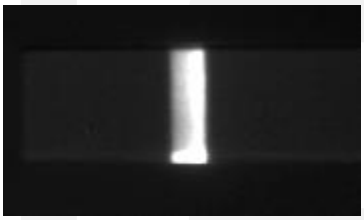
[AN] RTV-2023-0301-001

Application:

There are two types of lateral flow tests, one is the **colorimetric lateral flow test** and the other is the **fluorescent lateral flow test**. Unlike the colorimetric lateral flow test, in addition to requiring different light sources, fluorescent has a more uniform background, so the background causes less interference, as shown in the following figure:

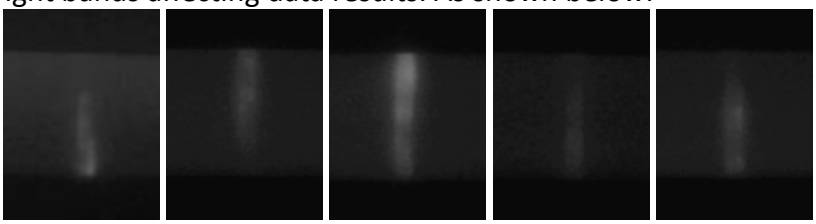
colorimetric lateral flow test

fluorescent lateral flow test



Therefore, when performing a fluorescent lateral flow test, some settings of reader will be different from the colorimetric lateral flow test. Adjustments to these settings can help:

1. Allow the Reader to detect more fluorescent signals.
2. Increase the intensity of the fluorescent signal.
3. Reduce the interference of other background signals.
4. Avoid fluorescence quenching
5. Even though the fluorescent lateral flow test (LFT)has higher sensitivity than colorimetric LFT, fluorescent labels such as latex beads are not as stable as the colorimetric labels. Avoid uneven bright bands affecting data results. As shown below:



Product:

RapidScan Lateral Flow Readers Pro with Rapid Test View (RTV)or Rapid Test View_Ethernet Software (ERTV)

Introduction:

When doing a **fluorescent lateral flow test (UV light)** through reader, the setting of **Profile Wizard** should be different from **colorimetric lateral flow test (white light)**. The differences are as follows:

1. Range setting of **C (control) & T (test) ROI**.
2. **Color Mode** setting.
3. Use **Standard Mode**.
4. Setting of **Integration Method**.

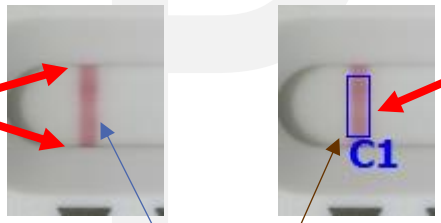
→ For details of the above settings, please refer to **Step**.

Steps:

1. ***Range setting of C (control) & T (test) ROI:***

A. **Colorimetric lateral flow test:**

- Edges cause shadows and affect calculation results, so this part needs to be avoided.



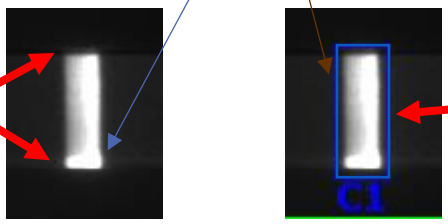
- The ROI width needs to be 10%~15% larger than the C/T bands width.
- The ROI height needs to be 15%-20% lower than the C/T bands. (15%~20% reduction of the top and the bottom)
- The shadows on the edges cause signal interference, the ROI height needs to be reduced to avoid detecting shadow signals. This is a compromise.

B. **Fluorescent lateral flow test:**

Band

ROI

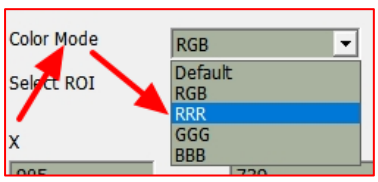
- There is no shadow problem at the edge, so this part of the signal can be included in the calculation result.

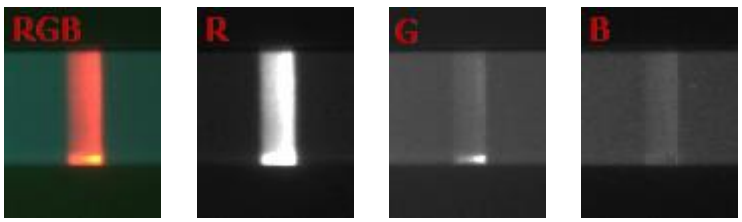


- The ROI width needs to be 15%~20% larger than the C/T bands width.
- The ROI height needs to be 15%-20% higher than the C/T bands. (Add 15%~20% of the top and the bottom)

2. ***Color Mode setting:***

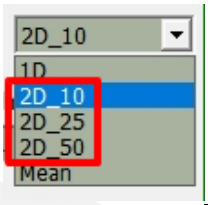
It is recommended to choose RRR so the signal is the strongest.





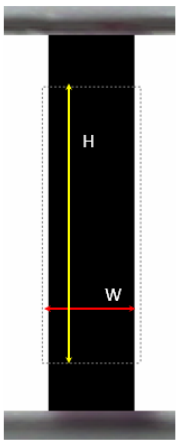
3. ***Setting of Integration Method:***

- It is recommended to choose 2D Integration Method (2D_10/2D_25/2D_50)
  → Because the fluorescent signal is very clear, the 2D Integration Method can fully present the signal value of each part in the ROI, so compared with 1D, 2D can improve the sensitivity.

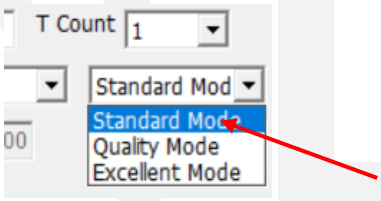


Integration Method

- **1D**: average each H and sum all W
- **2D**: sum all H x W pixels value = V_{2D}
  - 2D_10 = $V_{2D}/10$
  - 2d_25 = $V_{2D}/25$
  - 2d_50 = $V_{2D}/50$
- Value big→slop higher → noise high



4. ***Use Standard Mode:***



→ Avoid **fluorescence quenching**.

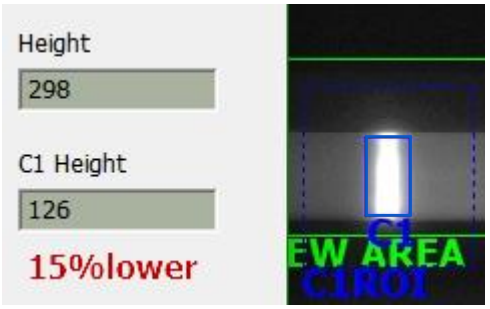
Example:

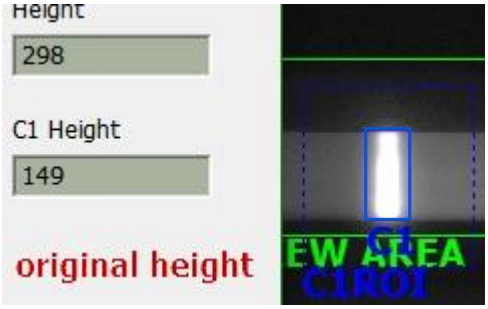
1. ***Experimental Content:***

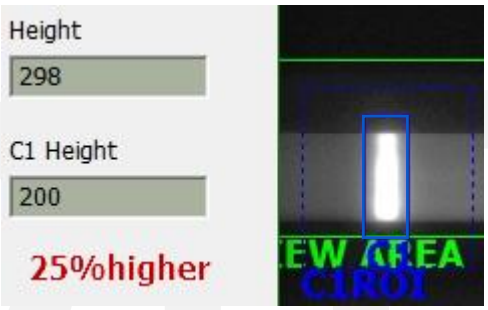
control variable	
Test sample	Sample A (fluorescent lateral flow test)
manipulative variable	
The height of ROI	15%lower original height 15%higher 25%higher
Integration Method	1D 2D_50
Strain variable	
Repeat the analysis of 13 samples to observe the numerical differences of different settings, and verify whether the settings mentioned by **Step** are appropriate.	

2. ***The height of ROI:***

A. ROI height data:







B. Use the **Batch Test** function, set the ROI height to the values in the above picture, re-analyze, and get the data as follows (For the "**Batch Test**" function, please refer to *[Application Note RTV-2022-1229-001] "Batch Test" Help Optimize the Profile Setting, the Test Images, and the Test Results*):

	original height		15%lower						15%higher						25%higher					
	Raw data		Raw data		The gap with the original height		Gap percentage		Raw data		The gap with the original height		Gap percentage		Raw data		The gap with the original height		Gap percentage	
No	C	T1	C	T1	C	T1	C	T1	C	T1	C	T1	C	T1	C	T1	C	T1	C	T1
1	28706	3055	25768	2800	2938	255	10.23%	8.35%	29305	3116	599	61	2.09%	2.00%	29615	3142	909	87	3.17%	2.85%
2	24499	2309	21719	2090	2780	219	11.35%	9.48%	25025	2379	526	70	2.15%	3.03%	25253	2401	754	92	3.08%	3.98%
3	13034	802	11367	663	1667	139	12.79%	17.33%	13338	850	304	48	2.33%	5.99%	13483	865	449	63	3.44%	7.86%
4	24399	9271	22167	8597	2232	674	9.15%	7.27%	24889	9485	490	214	2.01%	2.31%	25157	9515	758	244	3.11%	2.63%
5	23303	9109	20754	8152	2549	957	10.94%	10.51%	23870	9300	567	191	2.43%	2.10%	24145	9359	842	250	3.61%	2.74%
6	22905	8890	20588	8248	2317	642	10.12%	7.22%	23381	9033	476	143	2.08%	1.61%	23606	9053	701	163	3.06%	1.83%
7	23620	2460	21102	2194	2518	266	10.66%	10.81%	24107	2548	487	88	2.06%	3.58%	24310	2565	690	105	2.92%	4.27%
8	22442	2070	19930	1851	2512	219	11.19%	10.58%	22922	2134	480	64	2.14%	3.09%	23125	2142	683	72	3.04%	3.48%
9	20422	3596	18184	3319	2238	277	10.96%	7.70%	20850	3648	428	52	2.10%	1.45%	20987	3685	565	89	2.77%	2.47%
10	25802	3166	22885	2824	2917	342	11.31%	10.80%	26454	3231	652	65	2.53%	2.05%	26740	3264	938	98	3.64%	3.10%
11	19419	3779	17648	3696	1771	83	9.12%	2.20%	19866	3815	447	36	2.30%	0.95%	20078	3844	659	65	3.39%	1.72%
12	27313	0	24156	0	3157	0	11.56%	N/A	27918	0	605	0	2.22%	N/A	28147	0	834	0	3.05%	N/A
13	13333	210	11991	180	1342	30	10.07%	14.29%	13602	222	269	12	2.02%	5.71%	13676	219	343	9	2.57%	4.29%

C. The figure above shows:

In the data of **The Gap with the Original Height**, the settings such as **15%LOWER** have a large gap (relative to **15%Higher** and **25%Higher**).
-> If **ROI height** is reduced, there will be more signal loss and cause distortion. If the ROI height is increased, the data changes are not much because there is no noise interference value.
→ Therefore, it is recommended to increase the ROI height so that most signals can be detected.

3. ***Integration Method:***

Use reader to analyze different concentrations of sample A with different Integration Methods(1D & 2D_50), and record the T value results. The results are as follows:

	2D_50						1D					
	original height		15%higher				original height		15%higher			
	Raw data		Raw data		The gap with the original height		Raw data		Raw data		The gap with the original height	
No	C	T1	C	T1	C	T1	C	T1	C	T1	C	T1
1	28706	3055	29305	3116	599	61	9633	1025	8619	916	-1014	-109
2	24499	2309	25025	2379	526	70	8221	775	7360	699	-861	-76
3	13034	802	13338	850	304	48	4374	269	3923	250	-451	-19
4	24399	9271	24889	9485	490	214	8187	3111	7320	2789	-867	-322
5	23303	9109	23870	9300	567	191	7819	3056	7020	2735	-799	-321
6	22905	8890	23381	9033	476	143	7686	2983	6876	2656	-810	-327
7	23620	2460	24107	2548	487	88	7926	825	7090	749	-836	-76
8	22442	2070	22922	2134	480	64	7530	694	6741	627	-789	-67
9	20422	3596	20850	3648	428	52	6853	1206	6132	1073	-721	-133
10	25802	3166	26454	3231	652	65	8658	1062	7780	950	-878	-112
11	19419	3779	19866	3815	447	36	6516	1268	5843	1122	-673	-146
12	27313	0	27918	0	605	0	9165	0	8211	0	-954	0
13	13333	210	13602	222	269	12	4474	70	4000	65	-474	-5

The figure above shows:

When using 1D settings, increasing ROI will reduce the C/T value; the setting of 2D_50 has no such problem.

→ Integration Method recommends using 2D (including 2D_10/2D_25/2D_50) settings.