

Automatic inspection device for HCV antibody rapid test strips for the production line

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The paper presents an automatic inspection system for the hepatitis C virus (HCV) antibody rapid test strip, using computer vision device and new image processing algorithm. The rapid test strip contains a membrane strip which is pre-coated with HCV recombinant protein (NS3, NS4, NS5, core protein) on the test line region, which is on a fixed T line. One drop of specimen dilution is placed on the collection region. When specimen dilution rises to the T line position the system makes a colour judgment by digital CCD in a computer vision inspection system, without using human vision. Within 0.3 s after reaction, we can get a quantitative result. System operations are very simple and easy to use. Experiments were conducted to illustrate the comparison between this image-based system and many typical specimens with specific antigens that were prepared in the test assay configuration. This system can provide accurate measurement data in inspection within few minutes, and quality verification for the production line of rapid test strip is more convenient.

Keywords: Automatic inspection system, Digital CCD, Specific antigens

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Introduction

With advancements in biotechnology, visual inspection systems for rapid inspection test strips are increasingly important¹⁻⁴. Hepatitis C was caused by hepatitis C (HCV) virus. Its major routes of infection are through HCV infected blood or body fluid entering the human body by way of the skin or mucous membrane, finally entering blood and causing chronic hepatitis. As such, chronic hepatitis is often asymptomatic and is usually discovered by regular health examination. Hepatitis C can lead to a serious complications, causing patients to die with serious cirrhosis or hepatocellular carcinoma after 10-15 y. However, after a patient is infected by HCV, he can produce antibodies to resist intrusive HCV. The antibodies to HCV such as, IgG, IgM, IgA, are the main inspection objective of our proposed rapid test strip (Figure 1).

The study aims at producing an online quality

inspection method for rapid test strip, determining the best production parameters before manufacture and reducing the testing error to within 1 per cent. The optical portion of the vision system is a digital coupled-charge device (CCD) camera that has a dynamic image-grabbing device to acquire the rapid test strip image of the entire light field. This image is converted into a digital file by the image-grabber for computer analysis. A PC camera with USB 2.0 interface is fixed onto a frame to produce a suitable view angle of the tested object. There is no auto-gain control in CCD to avoid measurement errors and acquire the raw data, the tested object is placed onto a slide track, as shown in Figure 2.

The rapid test strips of our study apply the immunoassay technology and chromatographic method. They comprise a membrane strip which is

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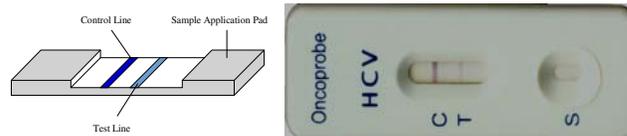


Figure1 – Outline of HC rapid test strip

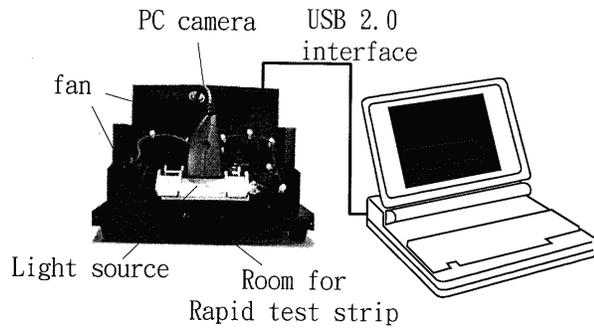


Figure 2 — Architecture of the digital image-based measurement system for rapid test strips

spray-coated with HCV recombinant protein (NS3, NS4, NS5, and core protein) on the test line region, which is on a fixed T line. We also use the Protein G coated particles as a colour displayer. Either serum or plasma could be the inspection specimen. After drawing blood and completing the centrifugal process the test can proceed immediately. During testing the specimen moves upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a colour line. Thus the presence of this colour line indicates a positive result, while its absence indicates a negative result. The specimen should be kept at 2~8°C for temporary storage, but it must be kept under -20°C for long-term storage.

Special properties of economic inspection device for rapid test strips are shown below:

- (i) When inspection specimen rises to the T line position, which takes approximately 10-15 min, it makes a colour judgment criterion by computer vision system. Thus, we can get a rapid quantitative result within 0.3 s.
- (ii) High sensitivity.
- (iii) Colour judgment criterion by computer vision system is highly reliable.
- (iv) Simple and easy to operate.

Software Calculation Method

In order to avoid subjective judgment error by different inspectors using human vision, we need quantitative inspection results. However, under the quantitative process of this economical inspection device, there are some problems that need to be solved, such as

- (i) Because the test line was made by roller smear the quality usually is not very uniform, causing blurring on the edge.
- (ii) Light must be obliquely incident to the test line, to show the inspection results. However, this increases the intensity distribution problem of the image. Thus, we use the light intensity of reference light-field measurement region as the basis for reference inspection.
- (iii) In order to firmly absorb the specimen the surface of the test strip is a special paper, which causes a dye effect for the inspection image and noise problem.

In order to measure the intensity distribution of the T line widely and effectively, the setting control bias for the light intensity is done in the image processing procedures. For this, we divided the bias adjustment into four levels so that the total scale will be 256 for brightness. The gray scale characteristic of the CCD camera has been calibrated by a photometer, so that a gray level of each light spot on the plate $g(x,y)$ can be transferred to an intensity value $f(g(x,y))$ from a photometer, where the function f is nonlinear. Hence the intensity measurement of the guide plate is acquired by grabbing the image off the plate with the calculations performed automatically by computer⁵.

Here, we can define probability level as:

$$P(x,y) = P_0 - f(g(x,y)),$$

where P_0 is a constant.

The light field measurement is an automatic inspection procedure and is controlled by our software programs. The designed software program outputs diagrams such as the T line intensity, the 3-D probability level $P(x,y)$ mesh diagram and automatic inspection for the image of rapid test strip. A positive reaction image of HCV rapid test strip and its 3-D probability graph are shown in Figure 3(a) and 3(b), respectively. Negative results are shown in Figure 4(a) and 4(b) respectively. Both Figure 3(b) and 4(b) seem same, but stand for positive and negative cases, so it needs more precise calculations which will be discussed as follows.

Figure 5 shows the lighter colour image of positive reaction. Integrating the gray level value of image along the vertical direction, then setting the threshold value of response over 500 by means of forward and backward difference method, we obtain the wave pattern (Figure 6). If we reduce the

threshold to 200, its response pattern is shown in Figure 7, which reveals the dye effect of inspection image and brings out noise persecution.

The mean value of multiple image gray level is shown in Figure 8. It can be observed that the suppressive effect for image noise is still very limited. So it is difficult to find the difference between Figure 4(a) and 4(b).

An image A , which translates x , can be defined by

$$(A)_x = \{c | c = a + x, a \in A\}.$$

The operation of gray-level image A that is etched by B can be represented by

$$(A \ominus B)(s, t) = \min\{A(s+x, t+y) - B(x, y) | (s+x),$$

$$(t+y) \in D_A; (x, y) \in D_B\},$$

where D_A and D_B are the domains of A and B , respectively; B is a filtering function of morphological

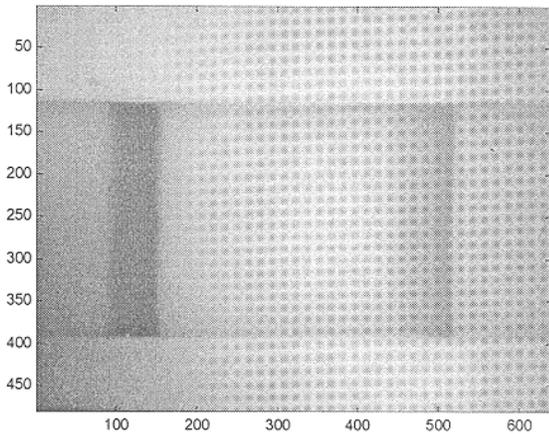


Figure 3(a) — Positive reaction image of an HCV rapid test strip

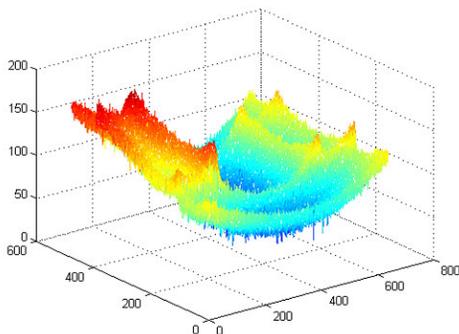


Figure 3(b) — 3-D probability graph for the image of a positive reaction

process; and $(s+x)$ and $(t+y)$ are the displacement parameters.

If we simplify the above formula to be one variable function, it becomes

$$(A \ominus B)(s) = \min\{A(s+x) - B(x) | (s+x) \in D_A; x \in D_B\}.$$

Let B be a 15×50 pixels mask and make a large range filter (5×200 pixels) for inspection image A . This mask uses an erosion operation to exclude noise, then integrates the gray level value of the image along the vertical direction and sets the threshold value of response higher than 500 by means of the forward and backward difference method. We can then obtain the filtering wave pattern, as shown in Figure 9, which shows the edge position of the T line and C line.

In the T line and C line, we can define probability density $P_n(G)$

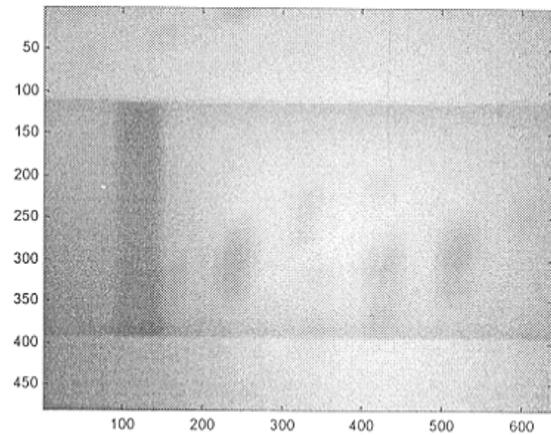


Figure 4(a) — Negative reaction image of an HCV rapid test strip

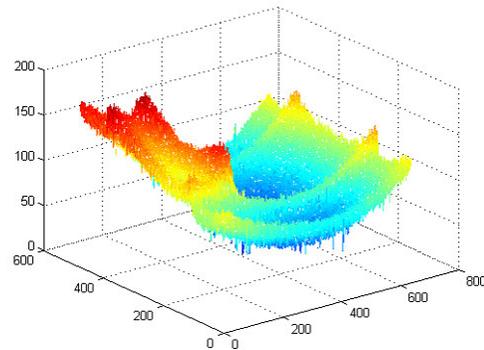


Figure 4(b) — 3-D probability graph for the image of a negative reaction

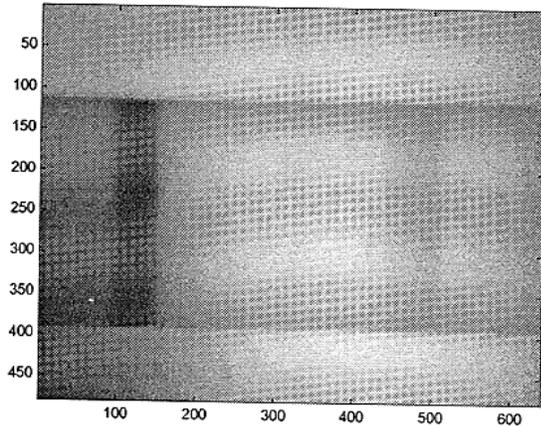


Figure 5 — Light colour image of a positive reaction

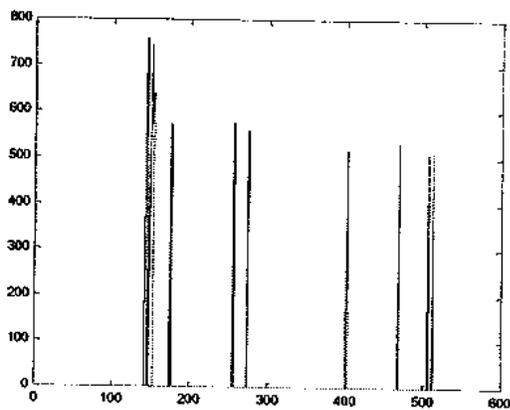


Figure 6 — Wave pattern where the threshold value of response is over 500

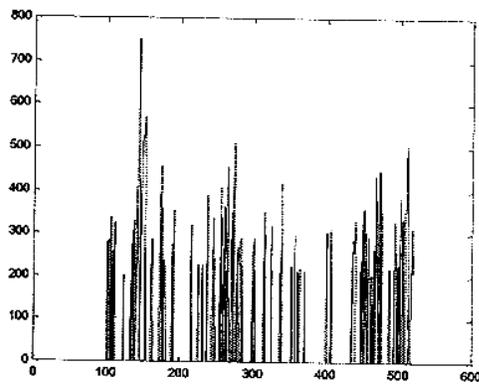
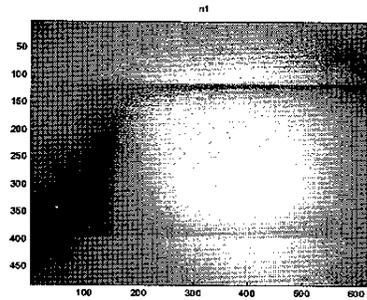


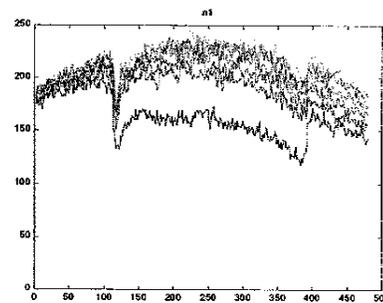
Figure 7 — Wave pattern the threshold value of response is reduced to 200

$$P_n(G) = \frac{N}{M} \leq 1,$$

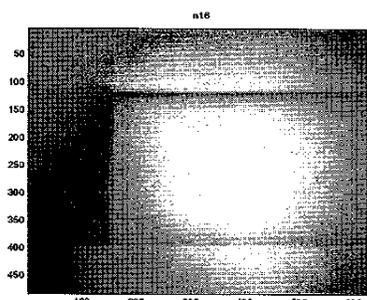
where G is the gray level, N is the pixel number of gray level G , and M is the pixel number of T line and C line.



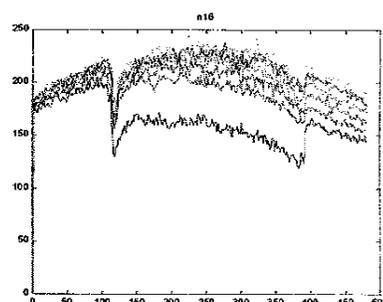
(a)



(b)



(c)



(d)

Figure 8 — Mean value of the multiple image gray level

From the calculations of $P_n(G)$ of first order statistical parameters, we can then use skewness ratio and kurtosis ratio to evaluate the spraying effect of T line and C line in the production line:

Skewness ratio =

$$\left[\frac{1}{S\sigma_n^3} \int (n - \mu_n)^3 p_n(n) dn \right] = \overline{(n - \bar{n})^3} / (S\sigma_n^3),$$

kurtosis ratio =

$$\frac{1}{K} \left[\frac{1}{\sigma_n^4} \int (n - \mu_n)^4 P_n(n) dn - 3 \right] = [\overline{(n - \bar{n})^4} / \sigma_n^4 - 3] / K,$$

where $\sigma_n = \sqrt{\int_n (n - \mu_n)^2 p_n(n) dn}$,

$$\mu_n = \int_n n p_n(n) dn,$$

and S, K are constants.

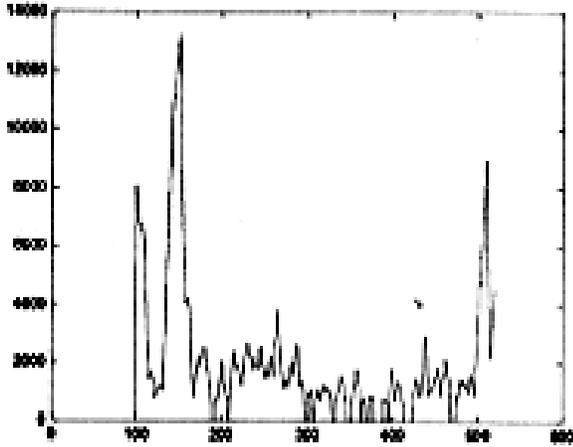


Figure 9 – Wave pattern of forward and backward difference method after filtering (threshold value 500)

Design for Image Processing Interface

In this practical design for the image processing interface, we divide the structure into two stages. The first stage is, designing a block chart which is based on the direct show structure, as shown in Figure 10.

The second stage is to design a component model, which is based on VCL structure, using C++ Builder, as shown in Figure 11. Using the VC++, functional interaction correlation between components of DirectShow and VCL can operate smoothly. Then the follow-up program can be developed by means of this VCL component, and can completely accomplish the machine vision component because it includes complete method, attribution and event functions. The attribution functions includes a capture card for the driver image, image width, colour adjustment and dialogue frame^{6,7}. The method functions include dialogue frame for start, stop and call; inside-build

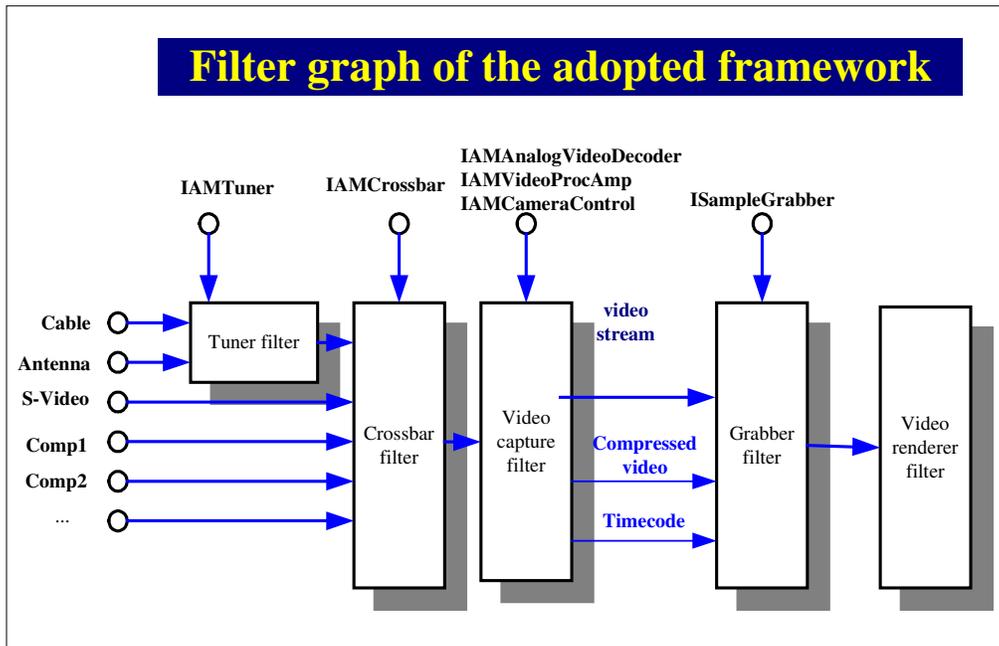


Figure 10 — Frame work of extensive core model for machine vision component

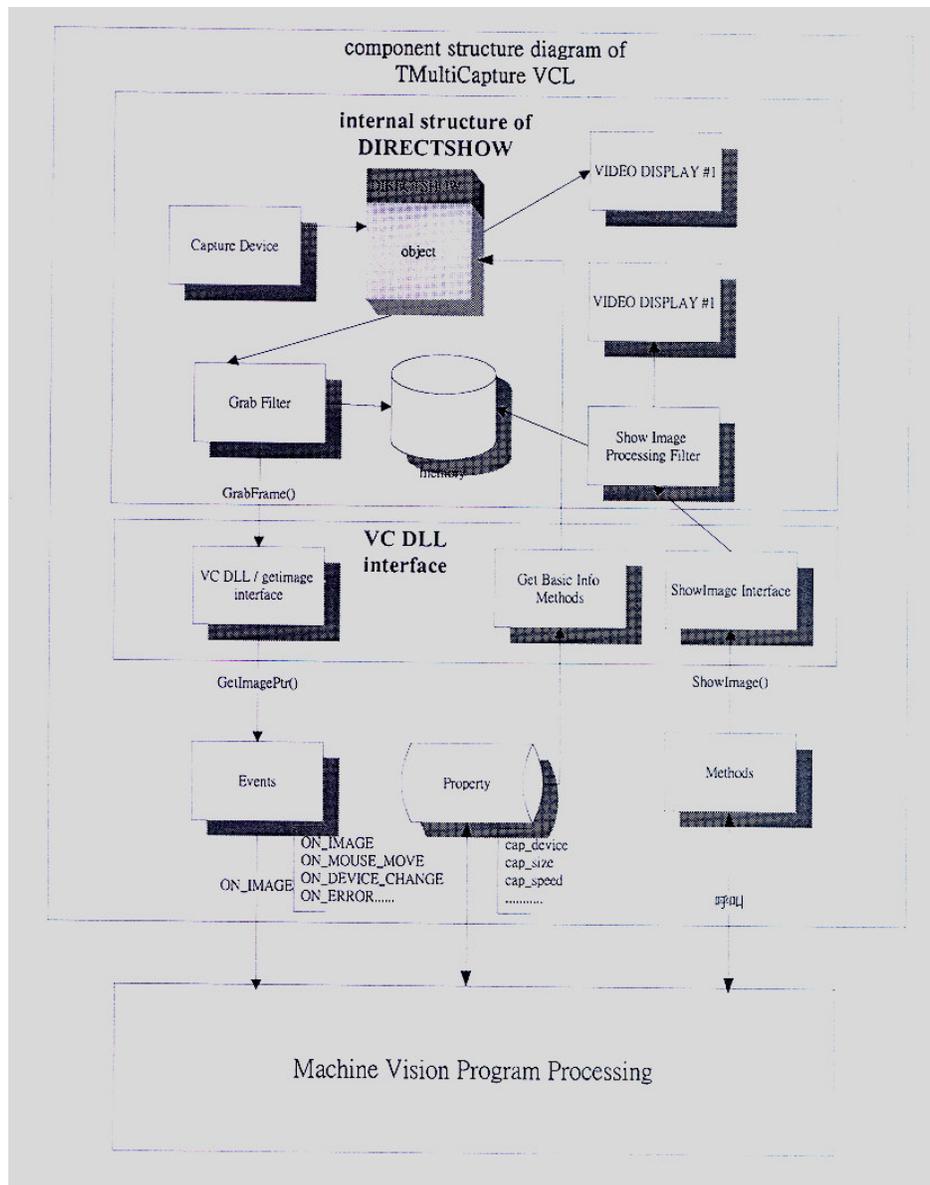


Figure 11 — Framework of the extensive machine vision model

colour procession; character display; and drafting; thus it can obtain all of the capture card attribution. The event functions include callback capability which can feedback events as it receives every image, mouse response and correction of element fundamental attribution, etc.

The key point of the overall system is that it can record the detailed capture data to a databank for future use and the completeness of the system parameters are the important setting point as it captures image.

Inspection Processes of the HCV Rapid Test Strip

The inspection processes of the proposed HCV rapid test strip are shown subsequently:

- (i) Remove the rapid test strip from the sealed foil pouch.
- (ii) Drop 30 μ L patient serum or plasma specimen onto the collection region, and add 3 drops dilution (about 90 μ L) to it.
- (iii) Wait for 10 to 15 min until the specimen dilution rises to the T line position.

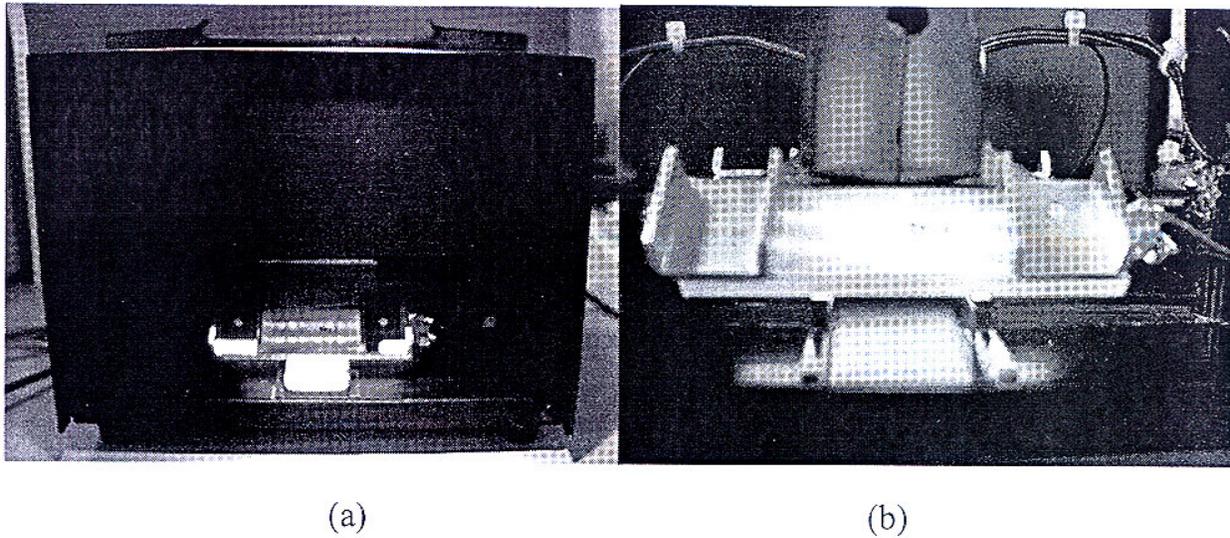


Figure 12 — Open sliding door of the economic inspection device for rapid test strips: (a) with housing and (b) Without housing

Table 1 — Test results from samples

Level	Reaction	Probability level	Skewness evaluation	Kurtosis evaluation
0	Negative	0	Symmetrical	Normal
10	Positive	24	Symmetrical	Normal
25	Positive	62	Unsymmetrical	Peaky
50	Positive	121	Symmetrical	Normal
75	Positive	173	Symmetrical	Normal

(iv) Put the rapid test strip into the inspection device, and then read the results [Figure 12(a)]. The light sources are two light guide plates which have uniform light density and distribution.

There are three kinds of inspection results.

(a) Positive Reaction

Both the *T* line and *C* line appear as coloured bands, even though the *T* line may appear lighter or darker than the *C* line with the light source coming from both sides of the backlight module [Figure 12(b)]. This indicates that the tester’s serum or plasma has an HCV antibody.

(b) Negative Reaction

One coloured band is in the *C* line but no coloured band is in the *T* line. This indicates there is no HCV antibody in the specimen.

(c) Invalid

If there is no coloured band or no coloured band in the *C* line the test result is invalid. This may be because rapid test strip is invalid or is beyond the expiration data or there has been improper operation. In this case the specimen is retested with a new rapid test strip.

Using the system software the image is received by a computer through an USB 2.0 transmission interface (transmission rate of 480 Mbps). Then, using the automatic vision inspection rule developed here, we can identify the biochemical reaction immediately, in order to determine if the reaction is positive or negative. If reaction is positive, inspector can calculate the grade of reaction concentration and provide objective and accurate data for a doctor to diagnose Hepatitis C. In this process the technician only needs to put the rapid test strip into the

inspection device, and push a start button. The system then identifies the biochemical reaction automatically, printing out a list and indicating the position of the positive reaction. Table 1 is an example of the test results from samples which shows the experiments which were conducted to illustrate the comparisons between this image-based system and many typical specimens with specific antigens that were prepared in the test assay configuration. To measure the quality of spraying effect of *T* line and *C* line, skewness and kurtosis evaluations are made, according to the skewness and kurtosis ratio parameters that are normalized with respect to the width. If the distribution of gray level is symmetrical the skewness ratio is close to zero. If there is no peakedness of the the distribution of gray level, the kurtosis ratio is also close to zero.

Conclusion

This paper describes an automatic inspection system for rapid test strips, using computer vision to promote the inspection accuracy and objectivity. This system can provide accurate measurement data in inspection within a few minutes. These data can provide convenient quality verification in the production line of the rapid test strip. It is also a very useful tool for patient management and disease control.

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References

- 1 Morozov V N, Gavryushkin A V & Deev A A, Direct detection of isotopically labeled metabolites bound to a protein microarray using a charge-coupled device, *J Biochem Biophys Methods*, **51** (2001) 57-67.
- 2 Song J M, Mobley J & Tuan V D, Integrated circuit microchip system with multiplex capillary electrophoresis module for DNA analysis, *Analyt Chim Acta*, **466** (2002) 187-192.
- 3 Song J M, Mobley J & Tuan V D, Detection of bacterial pathogen DNA using an integrated complementary metal oxide semiconductor microchip system with capillary array electrophoresis, *J Chromatogr B*, **783** (2002) 501-508.
- 4 Battistoni C, Kaciulis S, Mattogno G & Righini G, Noise removal from auger images by using adaptive binomial filter, *J Electron Spectros Relat Phenomena*, **76** (1995) 399-404.
- 5 Lin C S, Wu W Z, Lay Y L & Chang M W, A digital image-based measurement system for a LCD backlight module, *Optics Lasers Technol*, **33**(7) (2001) 523-529.
- 6 Lay Y L & Lin C S, Lens distortion correction by adjusting image of calibration target, *Indian J Pure Appl Phys*, **40** (2002) 770-774.
- 7 Lin C S, Lay Y L, Huan C C, Chang H C & Hwang T S, The preprocessing and recognition methods of an integrated automated production lot number inspection system, *J Sci Ind Res*, **62** (2003) 573-582.