Abstract: An automated cell staining apparatus comprises a chamber enclosing a microscope slide having a sample thereon, a steam heat source and an air source. The steam heat source is capable of heating the chamber, microscope slide and sample with steam to a desired temperature. The air source is capable of cooling the steam from the steam heat source with air for controllably reaching the desired temperature. In some embodiments, there is a temperature sensor for detecting the temperature and a controller programmed to cause the chamber to meet a desired set point temperature by supplying air and/or steam.
ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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CELL STAINING WITH AIR QUENCHED STEAM HEATING

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Applications No. 61/331,329, filed May 4, 2010, and 61/469,680, filed March 30, 2011, which are incorporated herein by this reference.

FIELD

This application relates to cell staining technology, and in particular to automated cell staining equipment and methods.

BACKGROUND

Cell staining methods, including immunostaining and in situ DNA analysis, are useful tools in histological diagnosis and the study of tissue morphology. Immunostaining relies on the specific binding affinity of antibodies with epitopes in tissue samples, and the increasing availability of antibodies which bind specifically with unique epitopes present only in certain types of diseased cellular tissue. Immunostaining requires a series of treatment steps conducted on a tissue section mounted on a glass slide to highlight by selective staining certain morphological indicators of disease states. Typical steps include pretreatment of the tissue section to reduce non-specific binding, antibody treatment and incubation, enzyme labeled secondary antibody treatment and incubation, substrate reaction with the enzyme to produce a fluorophore or chromophore highlighting areas of the tissue section having epitopes binding with the antibody, counterstaining, and the like. Each of these steps is separated by multiple rinse steps to remove unreacted residual reagent from the prior step. Incubations are conducted at elevated temperatures, usually around 40°C, and the tissue must be continuously protected from dehydration. In situ DNA analysis relies upon the specific binding affinity of probes with unique nucleotide sequences in cell or tissue samples and similarly involves a series of process steps, with a variety of reagents and process temperature requirements.
Current cell staining technology includes the Ventana BenchMark ULTRA, which is a fully automated instrument capable of processing 30 independent samples simultaneously. The BenchMark ULTRA system covers the tissue sample by continually applying the reagent until surface tension is overcome and the entire slide is covered. With this method, it takes about 600 microliters (µl) of reagent to achieve coverage. The 600µl solution contains 100µl of active ingredient and 500µl of buffer included to assure complete coverage of the slide. Mixing and constant interaction between the sample and the reagents is achieved by two opposing and tangential air jets which produce a mixing vortex. The current method of heating for the incubation period is through conduction using an electrical heating plate. A layer of oil on top of the reagents can be added to address evaporation.

The present apparatus and methods seek to advance and improve upon those in current use.

SUMMARY

Described below are various embodiments of an enhanced cell staining apparatus and associated methods that address some of the shortcomings in conventional approaches.

According to one implementation, an automated cell staining apparatus comprises a chamber enclosing a microscope slide having a slide thereon, a steam heat source and an air source. The steam heat source is capable of heating the chamber, microscope slide and sample with steam to a desired temperature. The air source is capable of cooling the steam heat source with air for controllably reaching the desired temperature.

The steam heat source is capable of heating the microscope slide and sample to a desired temperature between 35°C to 85°C. The apparatus is capable of maintaining the desired temperature within 1°C over a duration of testing. The apparatus is capable of maintaining different points across the microscope slide within 1.25°C of the desired temperature. The steam heat source is capable of heating the sample to the desired temperature within about three minutes, and more preferably, within about two minutes.
The steam heat source can include a flash boiler connected to a source of water and capable of heating water to steam by resistive heating. The steam heat source can include a spider valve positioned upstream from the flash boiler and downstream from the source of water.

The apparatus can include a controller programmed to heat the slide quickly using a first stage combination of steam and air to a temperature close to but less than the desired temperature followed by a second stage combination of steam and air to heat the slide to the desired temperature with minimal overshooting. The air source can be controlled by causing an air valve to pulse open and closed.

The steam can be directed in a substantially laminar flow and to heat the slide and sample through condensation heating. The slide can be positioned substantially level, and the source of steam heat can be configured to direct steam substantially parallel to a lower surface of the slide. The apparatus can comprise a generally horizontal duct positioned beneath the microscope slide that is capable of guiding steam and air entering the chamber in a direction along a lower surface of the slide until the steam and air exit the duct and are caused to flow over an upper surface of the slide. The chamber can be insulated and generally sealed from external surroundings.

According to another embodiment, an automated cell staining apparatus comprises an insulated chamber, a steam heat source, an air source, a wye valve and a controller. The insulated chamber is dimensioned to enclose a microscope slide having a sample thereon and comprises a steam and air inlet in a lower surface, a duct leading upwardly from the steam and air inlet and having a horizontal portion along a lower surface of the microscope slide, a vent in an upper surface of the chamber, and a temperature sensor capable of sensing the temperature in the chamber. The steam heat source is capable of supplying steam to heat the chamber, the microscope slide and the sample to desired temperatures by condensation heating, and comprises a flash boiler powered by cartridge heaters and a controllable steam valve. The air source is capable of generating air at a temperature cooler than the steam, the air source comprising an air pump and a controllable air valve. Steam from the flash boiler and the controllable steam valve, and air from the air pump and the controllable air valve, are combined at the wye valve and fed to the air and steam
inlet of the chamber, the resulting mixture having a temperature less than a
5 temperature of the steam. The controller is connected to the temperature sensor, the
controllable steam valve and the controllable air valve. The controller is
programmed to cause the chamber to reach a desired set point temperature by
actuating the controllable steam valve to supply more steam if a current temperature
sensed by the temperature sensor is below the desired temperature and by actuating
the controllable air valve to supply more air if the current temperature sensed by the
temperature sensor is above the desired temperature.

The foregoing and other features and advantages will become more apparent
from the following detailed description, which proceeds with reference to the
accompanying figures.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 is a schematic view of a control volume that represents a chamber of
the enhanced cell staining apparatus.

Fig. 2 is a drawing showing a representative velocity profile of a flow into
the chamber.

Fig. 3 is another schematic control volume representing the chamber with
steam and air inputs.

Fig. 4(a) is a schematic view showing liquid film condensation on a surface.
Fig. 4(b) is a schematic view showing dropwise condensation on a surface.
Fig. 5 is a schematic view showing various fluid flow regimes for
condensation on the outer surface of a slide as the thickness of the condensation
increases.

Fig. 6 is a perspective view showing an embodiment of a horizontal duct for
positioning beneath the microscope slide.

Fig. 7 is a perspective view of an oscillating platform for holding a
microscope slide and mixing liquids applied to the slide according to a first
approach.

Fig. 8(a) is a second alternative mixing approach of a translating head that
drags reagent across the slide through capillary action at a gap between the head and
an upper surface of the slide.
Fig. 8(b) is a third alternative mixing approach of a shaped cap that is caused to rock on a slide, thereby causing liquid on the slide to be mixed.

Fig. 9 is a Pugh matrix showing weightings for various test scenarios.

Fig. 10 is a perspective view of the overall testing apparatus.

Figs. 11(a)-11(d) are various views of a chamber within which the translating gap assembly and microscope slide are placed to isolate them from the exterior surroundings.

Fig. 12 is a cross-sectional view in elevation of the chamber showing paths for the steam/air mixture and its path of flow over the microscope slide.

Figs. 13(a)-13(g) are various views of a translating gap head.

Fig. 14(a) is a front elevation view of a translating gap head in position over a microscope slide.

Fig. 14(b) is an enlarged cross-sectional view in elevation showing the gap between the translating gap head and the microscope slide at a point spaced away from the mixing projection.

Fig. 14(c) is a cross-sectional view in elevation showing the smaller gap between the mixing extension on the translating gap head and the microscope slide.

Fig. 15 is a perspective view showing the motion of the translating gap head relative to the microscope slide.

Figs. 16(a)-16(g), Figs. 17(a)-17(c), and Figs. 18(a)-18(f) are various views of an alternative embodiment of a translating gap head that provides for removal of liquid from the slide by suction as well as supply of liquid to the microscope slide.

Fig. 19 is a perspective view of a linear guide screw powered by a stepper motor for moving the translating gap head.

Figs. 20 and 21 are elevation views of the linear guide screw and translating gap head in operation.

Figs. 22(a)-22(c) are various views of the flash boiler.

Fig. 23 is a perspective view of a portion of the system showing the chamber and the air and steam inputs to the chamber.

Fig. 24(a) is a schematic showing the controller and the connections to various other elements of the system.
Fig. 24(b) is another schematic view of the controller and connections to other components of the system.

Fig. 25 is a system controls and integration diagram.

Fig. 26(a) is a graph of temperature versus time for eight different combinations of steam and air.

Fig. 26(b) is a table of the supporting data for Fig. 26(a).

Fig. 26(c) summarizes air delay values and steam delay values for the respective combinations.

Fig. 27(a) is a graph of temperature versus time showing the time required to reach three different set point temperatures.

Fig. 27(b) is a table of the supporting data for the graph of Fig. 27(a).

Fig. 28(a) is a perspective view of the position of sensors on a slide to test for temperature uniformity.

Fig. 28(b) is a graph of temperature uniformity results.

**DETAILED DESCRIPTION**

Described below are improved cell staining systems and methods that are automated, thermally isolated, and capable of extension into a multi-cell commercial instrument. The new systems and methods offer several advantages, such as, e.g., providing faster heating, improving temperature uniformity, providing better mixing, providing evaporation control and/or reducing required reagent volumes.

The system performs basic functions necessary for the cell staining process. The system can reach a specific set-point temperature on a microscope slide between 35-85°C and maintain that temperature with no more than a 1°C variation relative to the set-point temperature. The system should also maintain temperature uniformly across the microscope slide with no more than 0.5°C variation from one portion of the slide to another. This tight tolerance ensures that the entire tissue sample is stained evenly. The system should be able to reach any set-point temperature in less than 3 minutes, which is the time required by the current Benchmark ULTRA. The system should eliminate evaporation of reagents from the microscope slide during the incubation period. The system should completely cover the microscope slide with reagents in order to insure that the entire tissue sample is covered. The system
should then thoroughly remove reagents from the microscope slide. Preferably, the system is fully automated.

In some implementations, a conventional reagent dispensing carousel is envisioned. For these implementations, reagent is generally applied from the top of the slide. The present assignee's U.S. Patents No. 5,595,707, No. 6,352,861, No. 6,827,901 and No. 6,943,029, which are incorporated herein by reference, are some examples of conventional approaches.

Currently, one conventional carousel dispenses reagents in 100μl increments. Thus, it would be beneficial for the new system to achieve complete slide coverage as well as optimal reagent-tissue interaction at this same 100μl volume constraint. Desirably, the method of heating the slide in the new system utilizes steam. The presence of steam affects many design aspects, including venting, drainage, size and corrosion. Removal of reagents from the slide in the new system is desirably through vacuum aspiration. Thus, the system should accommodate a suction apparatus above the slide together with a passageway for waste disposal, such as through suitable tubing. For a multi-slide processing instrument, each slide can be accommodated in an adiabatic chamber to prevent temperature influence on neighboring slides.

Theory and Equations

This section is a compilation of the background information found through research in the areas of thermodynamics, fluid dynamics, and heat transfer, which is presented here as a partial theoretical basis for the approach and results. The equations and theoretical concepts were considered during all stages, but the actual design, build and testing of the system described below was largely empirical. Further development of the design into an advanced medical diagnostic instrument for commercial sale is informed by continued consideration of these concepts.

Energy Analysis of the Chamber

The nozzle that releases steam-air mixture to the chamber operates for long periods of time under the same conditions. There will be a transient period where the slide reaches a certain temperature and the heat transfer rate to the slide becomes
steady. Therefore, there is no change in intensive or extensive properties in the control volume region. This means that the volume, mass and the total energy content must remain constant. There is no boundary work. The total mass or energy entering the system should be equal to the total mass or energy leaving the system.

For purposes of the fundamental heat transfer and fluid dynamics equations, the chamber for each slide can be illustrated as a control volume, e.g., as shown in Fig. 1.

The first law of thermodynamics states that energy can be transformed (changed from one form to another), but cannot be created or destroyed. Thus, the difference of an amount of energy entering a system and amount of energy leaving the system has to be energy change in the system. This can be negative or positive, i.e., cooling can be accomplished by losing energy and heating can be obtained by receiving energy from the outside environment. Thus, the energy balance for closed systems is expressed as:

\[ E_{in} - E_{out} = \Delta E_{sys} \]  
\[ \dot{E}_{in} = \dot{E}_{out} \]  

Similarly, the mass flow rate \( \sum_{in} rh = \sum_{out} rh \)  

The total amount of energy entering the system is equal to the total amount of energy leaving the system.

\[ \dot{Q}_{in} + \dot{W}_{in} + rh9_{in} = \dot{Q}_{out} + \dot{W}_{out} + \dot{m} \theta_{out} \]  
\[ \dot{Q}_{in} + \dot{W}_{in} + rh \left( h + \frac{v^2}{2} + gz \right) = \dot{Q}_{out} + \dot{W}_{out} + rhh + \frac{v^2}{2} + gz \]

The chamber receives energy from the outside environment via tubing. The chamber is assumed to be an ideal case, and thus it is assumed that there is no heat loss through the insulation. The weights of the components of the chamber parts (Fig. 10) are obtained from CAD (Solidworks). The energy can be expressed in terms of mass, specific heat and the temperature change of the system. To raise the
temperature of the system by one degree, the required energy to raise each of the individual components of the chamber is calculated as follows.

\[ c_{p,ABS \text{ plastic}} = 1.2 \begin{array}{l}
\frac{J}{g\text{K}} 
\end{array} \quad [4] \quad c_{p,\text{glass}} = 0.84 \begin{array}{l}
\frac{J}{g\text{K}} 
\end{array} \quad Q = m_c p \Delta T \quad (1.6) \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Q(J)</th>
<th>m(g)</th>
<th>( c_p \left( \frac{J}{g\text{K}} \right) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner chamber</td>
<td>190.51</td>
<td>158.76</td>
<td>1.2</td>
</tr>
<tr>
<td>Lower top</td>
<td>32.66</td>
<td>27.22</td>
<td>1.2</td>
</tr>
<tr>
<td>Glass slide</td>
<td>12.29</td>
<td>14.63</td>
<td>0.84</td>
</tr>
<tr>
<td>Translating gap head</td>
<td>2.72</td>
<td>2.27</td>
<td>1.2</td>
</tr>
<tr>
<td>Translating gap amount</td>
<td>5.45</td>
<td>4.54</td>
<td>1.2</td>
</tr>
<tr>
<td>Actuator</td>
<td>6.12</td>
<td>13.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Linear guide mount</td>
<td>8.14</td>
<td>9.07</td>
<td>0.897</td>
</tr>
<tr>
<td>Linear guide carriage</td>
<td>8.16</td>
<td>18.14</td>
<td>0.45</td>
</tr>
<tr>
<td>Linear guide</td>
<td>18.98</td>
<td>42.18</td>
<td>0.45</td>
</tr>
<tr>
<td>Linear guide screw</td>
<td>16.33</td>
<td>36.29</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Individual energies of each part of the chamber are summed to obtain the energy required to raise the temperature of the entire chamber.

\[ Q_{\text{total}} = 304.08/ \]

The time required to raise the temperature by one degree when a heat source of 1200 J is applied;

\[ E = 1200 \begin{array}{l}
\frac{J}{s} 
\end{array} \]

\[ \Delta T = \frac{\text{Total energy transferred}}{\text{Rate of Energy Transfer}} = \frac{E_{\text{in}}}{E_{\text{transfer}}} = \frac{304.08 \begin{array}{l}
\frac{J}{s} 
\end{array}}{1200 \begin{array}{l}
\frac{J}{s} 
\end{array}} = 0.25 \begin{array}{l}
s 
\end{array} \]

**Fluid Dynamics Analysis - Mass and Volume Flow Rates: Solenoid Valve Exit**

The mass flow rate can cause the temperature of the chamber to increase or decrease. If the energy input is less than the energy loss, the chamber temperature will decrease. Regulated mass flow rate determines the accuracy of the energy input. In fluid dynamics, a mixture travelling through a duct will have a zero
velocity at the solid wall. Considering a no slip condition at the walls, the velocity profile develops, concentrating in the middle, as shown in Fig. 2. The differential mass flow rate that flows through an area of \( dA_c \), relationship can be expressed as:

\[
\delta \dot{m} = \rho V_{or} A_{cross \, section} \quad (2-1)
\]

Integrating this equation,

\[
\dot{m} = \int_{A_c} P^{normal \, - \, cross \, section} \quad (2-2)
\]

With respect the accuracy of this equation, the mass flow rate can be expressed in terms of average values where the average velocity can be defined as:

\[
\dot{m}_{\text{average}} = \frac{1}{A_c} \int_{A_c} P^{normal \, - \, cross \, section} \quad (2-3)
\]

where

\[
\dot{m}_{\text{average}} = \text{the average velocity throughout the cross section of the nozzle}
\]

Thus, \( \dot{m} = p V_{\text{average}} A_{\text{cross \, section}} \quad (2-4) \)

Where

\( p = \text{density of the fluid} \)

Using this expression, the volume flow rate can be expressed as,

\[
\dot{V} = V_{\text{average}} A_{\text{cross \, section}} \quad (2-5)
\]

In order to determine the amount of air to be mixed with the water vapor, it is required to estimate the mass flow rate of the air and water released into the flash boiler. The velocity of the air exiting the nozzle was determined by experimental testing. The nozzle consists of a circular duct, fed by a motor. The experiments were performed at the exit of tubing where the air leaves into the atmosphere. A pressure transducer is a capacitance device in which the measured capacitance changes when the pressure on one of its side moves its diaphragm. It indicates the pressure in inches of water, which is a unit of choice when measuring small pressure differences. The readings indicated the pressure difference, \( P_s - P_f \), in inches of water, and these converted to Pascals per square inch. The experiments were performed using three different tubing lengths to note the pressure losses due to the length of the tubing.
Conversion factor: \( \frac{Pa}{in^2} = (in \times water) \times p_{H_2O} \times g \)

<table>
<thead>
<tr>
<th>Pressure (in H(_2)O)</th>
<th>Pressure (Pa)</th>
<th>Pressure (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.08</td>
<td>767.14</td>
<td>47.87</td>
</tr>
<tr>
<td>3.06</td>
<td>762.16</td>
<td>47.71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tube Length 12.4 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.06</td>
</tr>
<tr>
<td>3.05</td>
</tr>
<tr>
<td>3.05</td>
</tr>
<tr>
<td>3.05</td>
</tr>
</tbody>
</table>

| 2.73                  | 679.97       | 45.07          |
| 2.73                  | 679.97       | 45.07          |

<table>
<thead>
<tr>
<th>Tube Length 3.4 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.73</td>
</tr>
<tr>
<td>2.73</td>
</tr>
<tr>
<td>2.73</td>
</tr>
<tr>
<td>2.73</td>
</tr>
</tbody>
</table>

| 2.74                  | 682.46       | 45.15          |
| 2.73                  | 679.97       | 45.07          |
| 2.73                  | 679.97       | 45.07          |

<table>
<thead>
<tr>
<th>Tube Length 9.0 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.72</td>
</tr>
<tr>
<td>2.72</td>
</tr>
<tr>
<td>2.72</td>
</tr>
</tbody>
</table>

It can be noted that the average velocity leaving the nozzle is 45 m/s.

Recalling the formula: \( m = pAV \)
\[ V = 4.5 \frac{m}{s} \quad p = 1.087 \frac{\text{g}}{\text{m}^3} \quad d = 2.2 \text{ mm} \]
\[ A = \pi (1.1 \times 10^{-3} \text{m})^2 = 3.8 \times 10^{-6} \text{m}^2 \]
\[ rh = pAV = \left(1.087 \frac{\text{g}}{\text{m}^3} \right) (3.8 \times 10^{-6} \text{m}^2) \left(4.5 \frac{\text{m}}{\text{s}}\right) = 0.000146 \frac{\text{kg}}{\text{s}} \]

**Applying Principles to the Boiler Inlet: Water Flow through the Nozzle**

The nozzle inlet at the flash boiler feeds the boiler with water. The weight of the water dispensed from the nozzle is obtained using an analytical balance and dividing by the time to obtain the mass flow rate.

**Assumptions:**
1. Water is an incompressible substance
2. Flow through the nozzle is steady with time increments

**Properties:**
\[ m = f \]  
(2.6)

<table>
<thead>
<tr>
<th>Time, ( t ) (s)</th>
<th>Weight, ( m ) (g)</th>
<th>Mass Flow Rate, ( \dot{m} ) (g/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>12.4</td>
<td>1.24</td>
</tr>
<tr>
<td>20</td>
<td>26.1</td>
<td>1.305</td>
</tr>
<tr>
<td>30</td>
<td>38.6</td>
<td>1.286666667</td>
</tr>
</tbody>
</table>

*Average value is obtained to be 0.00127 \( \frac{\text{kg}}{\text{s}} \)*

**Water Vapor - Gas Mixture Analysis - Mixing Analysis: Steam and Air Ratio**

In this application, the steam at 100°C is to be mixed with air at 24°C. This occurs in the mixing chamber as shown in Fig. 3. Individual temperatures and mass flow rates of the air and water are used as an input to find the mixture temperature and mass flow rate. The mixing chamber is well insulated and does not involve any kind of work on the system. The kinetic and potential energies of the system are also negligible during the mixing process.
In Fig. 3, \( T_1 \) denotes steam at 100°C, and \( T_2 \) denotes air at 24°C being mixed in the chamber. The ratio of the mass flow rates of the respective \( T_1 \) and \( T_2 \) streams is required to obtain a desired output. The ratios are entered into the control system of the device.

5

Assumptions:
1. This is a steady state flow process and there is no change with time
2. The kinetic and potential energies are negligible, \( ke = pe = 0 \).
3. Heat loss from the system is negligible. \( (Q = 0) \).
4. There is no work interaction involved.

Analysis:

The mixing chamber is taken as the system. It is considered as the control volume because mass crosses through the system. There are two inlets and one outlet.

The mass balance can be written as:

\[
\dot{m}_{in} - \dot{m}_{out} = \frac{dm_{system}}{dt} = 0 \quad (3.1)
\]

\[
\dot{m}_{in} = \dot{m}_{out} \quad (3.2)
\]

\[
\dot{m}_1 + \dot{m}_2 = \dot{m}_3 \quad (3.3)
\]

Energy Balance:

\[
\dot{E}_{in} - \dot{E}_{out} = \frac{dE_{system}}{dt} = 0 \quad (3.4)
\]

\[
\dot{E}_{in} = \dot{E}_{out} \quad (3.5)
\]

Combining the mass and energy balances

\[
\dot{m}_1 h_1 + \dot{m}_2 h_2 = \dot{m}_3 h_3 \quad (3.6)
\]

Dividing the equation by \( rh_2 \) yields

\[
y h_1 + h_2 = (y + l)h_3 \quad (3.7)
\]

where \( y = \frac{m_1}{m_2} \)
The equation 3.7 provides the enthalpy of the mixture when the inputs are the atmospheric air and steam at 100°C. The ratios are further incorporated into the test plan and the control system.

5 Specific and Relative Humidity of Air

The amount of water vapor in atmospheric air supplied to the air-steam mixture can vary. It is not a fixed quantity and differs depending on the location and conditions of the weather. The amount of water in the air has to be determined theoretically in order to calibrate the device each time it starts the operation cycle.

The equations are used in the testing process of the device. The most proper way is to use mass of water vapor present in a unit mass of dry air. Absolute or specific humidity (denoted as $\omega$) can be expressed as following,

$$\omega = \frac{m_v}{m_a} = \frac{P_v/R_v T}{P_a/R_a T} = \frac{P_v}{P_a} = 0.622 \frac{P_v}{P_a} \quad (3.8)$$

or

$$\omega = \frac{0622R_v}{P - P_v} \quad (3.9)$$

where

$\omega_v = \text{water vapor (kg)}$

$m_a = \text{dry air (kg)}$

$P = \text{total pressure}$

Dry air contains no vapor. The specific humidity will increase as vapor is added. The specific humidity will increase until the air cannot accommodate more moisture. At this point the air is defined as saturated air. If moisture is added to the air, it will condense. The ratio of the amount of moisture the air holds ($m_v$) to the maximum amount of moisture that the air can hold at the same temperature ($m_g$) is called relative humidity (denoted as $\phi$). This requires a digital relative humidity measurement instrument. The key point is to standardize the water vapor-air ratio in atmospheric air taken into the system and avoid variation in specific humidity. We need to know the temperature that provides specific humidity.

$$\phi = \frac{m_v}{m_g} = \frac{P_v/R_v T}{P_g/R_v T} = \frac{P_v}{P_g} \quad (3.10)$$
where
\[ P_g = P_{sat@T} \]
Combining the equations
\[ \varphi = \frac{\omega \rho}{(0.622 + \omega)P_g} \]  
(3.11)
\[ \omega = \frac{0.622\varphi P_g}{P - \varphi P_g} \]  
(3.12)

Relative humidity ranges from 0 (dry air) to 1 (saturated air). The amount of moisture that dry air can hold depends on its temperature. Therefore, the device needs to be calibrated at the beginning of each operation cycle. The calibration will provide a reference point that the control system can calculate the ratio of steam to air to be mixed. The enthalpy of atmospheric air is the sum of the enthalpies of dry air and water vapor:
\[ H = H_a + H_v = m_a h_a + m_v h_v \]  
(3.13)
Dividing this equation by \( m_a \):
\[ h = h_a + \omega h_g \]  
(3.14)

Our system nozzle outputs \( rh = 0.000146 \frac{h_g}{s} \)

Assumptions: The dry air and the water vapor in the room are ideal gases. Relative humidity in Tucson is 30%. (Assumed value, the relative humidity in Tucson varies from 20% to 80%)

Properties: The constant-pressure specific heat of air at room temperature is
\[ C_p = 1.005 \frac{kJ}{kg \cdot K}. \]  For steam at 25°C, we have \( T_{sat} = 3.1698 \text{ kPa} \) \textit{and} \( h_g = 2546.5 \frac{kJ}{kg} \).

The partial pressure of dry air can be determined from
\[ P_a = P - P_v \]  
(3.15)
\[ P_a = \varphi P_g = \varphi P_{Sat@25°C} = (0.30)(3.1698 \text{ kPa}) = 0.951 \text{ kPa} \]
Thus,
\[ P_a = 101.325 - 0.951 \text{ kPa} = 100.374 \text{ kPa} \]

Specific humidity of air is determined from
\[ \omega = \frac{0.622P_v}{P - P_v} = \frac{(0.622)(0.951 \text{ kPa})}{(100.374)/cPa} = 0.005893 \frac{kg H_2O}{kg \text{ dry air}} \]
The enthalpy of air per unit mass of dry air is determined from
\[ h = h_a + \omega h_v \approx c_p T + \omega h_g = \left( \frac{1.005 \text{kJ}}{\text{kg} \cdot ^\circ C} \right) (25^\circ C) + (0.005893) \left( \frac{2546.5 \text{kJ}}{\text{kg}} \right) \]
\[ - \frac{40.131 \text{kJ}}{\text{kg dry air}} \]

**Additional Information:** Both the dry air and the water vapor fill the chamber completely. Therefore, the volume of each gas is equal to the volume of the chamber:
\[ V_a = V_v = V_{chamber} = 2.5 \text{ cm} \times 3 \text{ cm} \times 7 \text{ cm} = 52.5 \text{ cm}^3 = 52.5 \times 10^{-6} \text{ m}^3 \]
\[ m_a = \frac{P_a V_a}{R_a T} = \frac{(69.57 \text{ kPa})(52.5 \times 10^{-6} \text{m}^3)}{0.287 \text{ kPa} \cdot \frac{\text{m}^3}{\text{kg} \cdot \text{K}}(298\text{K})} = 0.0427 \text{ g} \]
\[ m_v = \frac{P_a V_a}{R_a T} = \frac{(30.426 \text{ kPa})(52.5 \times 10^{-6} \text{m}^3)}{0.4615 \text{ kPa} \cdot \frac{\text{m}^3}{\text{kg} \cdot \text{K}}(298\text{K})} = 0.0116 \text{ g} \]

The values above provide dry air - water vapor in the atmospheric air at room temperature operating conditions.

**Gas Vapor Mixtures: Approximation Enthalpy for Steam at 100°C**

The fluid mixture that is fed to the chamber consists of air at 24°C and steam at 100°C. Air is a mixture of mainly nitrogen and oxygen and some other gasses in small amounts. Air present in the atmosphere has some water vapor and called atmospheric air. However, if air contains no water vapor, it is called dry air. In this particular case, since the atmospheric air is used, the air can be considered as a mixture of dry air and water vapor. Assuming air temperature is 24°C, dry air can be considered as an ideal gas with a constant \( C_p \) value of 1.005 \text{kJ/kg \cdot K}. As the reference temperature taken as 0°C, the enthalpy change is the following,
\[ h_{dry air} = C_p T = \left( \frac{1.005 \text{kJ}}{\text{kg} \cdot ^\circ C} \right) T \left( \frac{\text{kJ}}{\text{kg}} \right) \]
Considering the water vapor in the atmospheric air, water vapor can be treated as an ideal gas. The ideal-gas relation can be applied to the water vapor in the air. Treating the atmospheric air as an ideal gas, the partial pressures of water vapor and dry air will give the total pressure.
If the water vapor is considered as an ideal gas, the enthalpy of water is a function of temperature only. Thus, enthalpy of water vapor in air is approximately equal to the enthalpy of saturated vapor at the same temperature.

\[ h_v(T, \text{low } P) \approx h_g(T) \]

The enthalpy of water vapor is 2676 kJ/kg. The average \( C_p \) value of water vapor at 100°C is 1.859 kJ/kg °C. The enthalpy of water vapor can be determined from the following equation.

\[ h_g(T) \approx 2676 + 1.859 \times (100°C) \text{ (kJ/kg)} \]

After the enthalpy values for atmospheric air and water vapor are obtained, the values are used in the equation with the mass flow rates. Thus, the enthalpy of the mixture is obtained.

\[
\hat{m}_{\text{atmospheric air}} = \frac{40.131}{k} \text{ kg/s}
\]

\[ h_{\text{steam @100°C}} = 2730.3 \text{ kJ/kg} \]

\[ \dot{m}_{\text{atmospheric air}} = 0.0000146 \text{ kg/s} \]

\[ \dot{m}_{\text{steam}} = 0.00127 \text{ kg/s} \]

\[ y h_1 + h_2 = (y + l) h_3 \quad (3.7) \quad \text{where} \quad y = \frac{\dot{m}_1}{\dot{m}_2} \]

The mass flow rates were modified in order to increase and decrease the enthalpy of the mixture. Once the enthalpy of the mixture is known, the temperature under a specific pressure (input) can be obtained from the psychrometric chart. For more complex systems, instead of the chart, a computer program can be written to obtain exact temperatures in order to eliminate error.

**Film and Dropwise Condensation on Horizontal Surfaces**

When the temperature of a vapor is lowered to \( T_{\text{sat}} \) condensation occurs in the chamber. Thus, we need to consider the heat transfer during the vapor-to-liquid phase transformations. Noting that under equilibrium conditions the temperature remains constant during a phase-change process at a fixed pressure, large amounts
of heat (due to the large latent heat of vaporization released) are transferred during condensation. Heat transfer coefficients associated with condensation are much higher than those encountered in other forms of convection and conduction. The water vapor contacts a solid surface with a temperature \( T_{\text{sat}} \) at below the saturation temperature \( T_{\text{sat}} \) of the vapor. The type of the condensation will depend on the velocity and the pressure of the steam-air mixture at the exit of the nozzle. There are two distinct forms of condensation.

1. **Film condensation**: The condensate wets the surface and forms a liquid film on the surface that slides down under the influence of the gravity (in our case the flow direction). The thickness of the liquid film increases in the flow direction as vapor condenses on the film as shown in Fig. 4(a).

2. **Dropwise condensation**: The condensed vapor forms droplets on the surface instead of a continuous film, and the surface is covered by countless droplets of varying diameters as shown in Fig. 4(b).

In film condensation, the surface is blanketed by a liquid film of increasing thickness, and this "liquid wall" between solid surface and the vapor serves as a resistance to heat transfer. The heat of vaporization \( h_{lv} \) released as the vapor condenses must pass through this resistance before it can reach the solid surface and be transferred to the medium on the other side.

In dropwise condensation, the droplets slide down when they reach a certain size, clearing the surface and exposing it to vapor. There is no liquid film in this case to resist heat transfer. As a result, heat transfer rates are 10 times larger than with film condensation. It is required to determine the Reynolds number in this case to find out whether the flow is laminar or turbulent.

\[
Re = \frac{D_h \rho_l V_l}{\mu_l} = \frac{4A_c \rho_l V_l}{p \mu_l} = \frac{4p V_l}{\mu_l} = \frac{4m}{p \mu_l} \quad (4.1)
\]

where

\[
D_h = \frac{4A_c}{V} = 45 = \text{hydraulic diameter of the condensate flow} \quad (4.2)
\]

\( p = \text{wetted perimeter of the condensate flow,m} \)

\( A_c = \rho \delta = \text{wetted perimeter} \times \text{film thickness, m}^2, \text{cross-sectional area of the condensate flow at the lowest part of the flow} \)
\[ \rho = \text{density of the liquid, } \frac{kg}{m^3} \]
\[ \mu = \text{viscosity of the liquid, } \frac{kg}{m \cdot s} \]
\[ V = \text{average velocity of the condensate at the lowest part of the condensate flow at the lowest part of the flow, } \frac{m}{s} \]
\[ \dot{m} = \rho V A_c = \text{mass flow rate of the condensate at the lowest part, } \frac{kg}{s} \]

To find the hydraulic diameter, the condensate in an actual condensation process is cooled further to some average temperature between \( T_{\text{sat}} \) and \( T_s' \), releasing more heat in the process.

Modified latent heat of vaporization is defined as:
\[ h'_{fg} = h_{fg} + 0.68 C_p (T_{\text{sat}} - T_s') \quad (4.3) \]
where
\[ C_p = \text{the specific heat of the liquid at the average film temperature} \]

The rate of the heat transfer can be expressed as
\[ \dot{Q} = h A_s (T_{\text{sat}} - T_s) = \dot{m} h'_{fg} \quad (4.4) \]

Therefore the Reynolds number can be written as:
\[ Re = \frac{4 \dot{Q}_{\text{conden}}}{\rho u_l h'_{fg}} = \frac{4 A_s (T_{\text{sat}} - T_s)}{\rho u_l h'_{fg}} \quad (4.5) \]

The properties of the liquid should be evaluated in the film temperature
\[ T_f = \frac{T_{\text{sat}} + T_s}{2} \]
which is approximately the average temperature of the liquid. However, \( h'_{fg} \) should be evaluated at \( T_{\text{sat}} \) since it is not affected by the subcooling of the liquid.

The Reynolds number for condensation on the outer surface of the slide increases in the flow direction due to the increase of the liquid film thickness, as shown in Fig. 5.

According the experiments, the liquid film remains smooth and wave free for \( Re < 10 \). Thus, the flow is clearly laminar. The condensate flow is called wavy laminar in the range of \( 450 < Re < 1800 \).
Then Newton's second law of motion for the volume element shown in Fig. 5 in the horizontal x-direction can be written as

$$\sum F_x = m a_x - \mu_i \frac{d u}{d y} (b dx) = 0 \quad (5.6)$$

$$ma_x = \mu_i \frac{d u}{d y} (b dx) \quad (5.7)$$

$$\frac{d u}{d y} = \frac{ma_x}{\mu_i (b dx)} \quad (5.8)$$

where \( b \) = width

$$\sum F_y = 0$$

**F_{downward} - F_{upward}**

weight = Buoyance force

$$Pi(5 - y)(b dx) = \rho_v (\delta y - y)(b dx) \quad (5.9)$$

$$Pi = P_v \quad (5.10)$$

To sum up, surface tension should be taken into account in the horizontal film condensation that takes place on the slide unlike the condensation on the horizontal walls of the chamber.

**Steam Heating**

Various designs for steam heating are described. These designs include vertical heating and horizontal flow heating. In the vertical heating case, the steam is dispensed from a nozzle located perpendicularly, centered below the slide. The steam flow makes contact with the bottom of the slide and heats through condensation phase change. The steam flow then fills the chamber where it creates a 100% humid atmosphere that prevents evaporation of reagents from the microscope slide. One problem discovered with the horizontal heating design is non-uniform temperature distribution across the microscope slide due to turbulence in the flow as it collides with the bottom surface of the slide and curls back. The second preliminary design for steam heating is a horizontal half-tubular flow guiding duct 200. This design, which is shown in Fig. 6, guides the steam flow in a laminar fashion beneath the microscope slide where condensation heating takes place. The steam flow is then guided to the top surface of the slide where it prevents
evaporation. The laminar flow should provide a much more uniform distribution of heat across the microscope slide.

**Spreading Reagent**

In order to spread the reagent, two designs were evaluated. A first design, named the "Hula" for its dancing Hula motion, comprises an oscillating platform to which a microscope slide is attached as shown in Fig. 7. The oscillation forces the applied reagents to overcome surface tension and cover the slide while maintaining reagent and tissue interaction. Preliminary test results showed inconsistent slide coverage, dry spots and regions not covered as desired.

A second proposed design, known as the translating gap, has a head 220 that translates across the slide 222 and causes reagent to be dragged across the slide through capillary attraction at a gap between the head and the slide, as shown in Fig. 8(a). A mixing tab may be located at the center of the gap head to create turbulence within the reagents. Turbulence increases interaction between the tissue sample and the reagents. The translating gap approach can effectively cover the slide with a small volume of reagent.

A Pugh matrix (Fig. 9) was constructed for all four design options with corresponding weights assigned to the contending design approaches. The final scoring from the Pugh matrix indicated that the horizontal method for heating along with the translating gap approach as for reagent application was the optimal design option.

Based on the Pugh matrix results, an integrated system of components needed to perform a standard cell staining process, as shown in Fig. 10. Tissue samples being tested are fixed to a microscope slide and placed in the adiabatic chamber to be stained. The major functional components include a translating gap assembly for spreading, mixing, and removing reagents. A flash boiler and an air pump are used for creating an air-steam mixture to heat the chamber. Two microcontrollers are used to automate the entire system.

Preliminary testing included narrowing down optimal speeds for gap translation (i.e., speeds of the head), reagent volumes, suction pressure, and steam flow characteristics. The optimal speed for gap translation was determined by
observing area covered and overspill as a function of speed of the translating gap head moving across the slide. Additionally it was found that minimal amounts of reagents could be used, far below 100µL. It was determined that a suction pressure above 5 psi is adequate for proper removal of the reagents. Expected information was obtained from the preliminary testing along with vital unforeseen discoveries. From these tests it was identified that the spray foam insulation had large thermal expansion characteristics which resulted in distortions of the chamber walls. Optimum chamber ventilation was determined along with increased chamber size for inner workings.

Adiabatic chamber

In order to achieve a specific set point temperature and reduce heat transfer to the external chamber environment, an insulated chamber 302 was designed. The chamber is made from ABS plastic with a dual wall construction. This dual wall construction was achieved by designing an inner shell 304 and outer shell 306, which allows for the insertion of an insulating material 308, as is shown in Figs. 11(a)-1 1(d). In this case, high density polystyrene foam insulation was selected because of its operating temperature range and ability to be easily cut.

In order to achieve uniform temperature distribution along the microscope slide while eliminating evaporation of the reagents, the chamber was designed with a flow guiding duct 308. As shown in Fig. 12, the flow guiding duct 308 directs the incoming steam/air mixture in a laminar fashion beneath the microscope slide. Here, the slide is heated through condensation heating which is kept uniform by the laminar flow, Fig. 12. The duct then directs the flow above the microscope slide where the steam/air mixture creates a 100% humid atmosphere which prevents the reagents from evaporating off of the microscope slide and tissue sample. The mixture is then guided to a vent 310 in the lid 312 of the chamber where it exits to the surrounding atmosphere. In order to assure that the steam/air mixture follows the desired flow path, weather stripping 314 and sealant was installed between the chamber lid interface and all chamber inlets and outlets, creating an air tight seal. This seal forces all flow entering the chamber to exit through the strategically placed vent 310.
The inner chamber is made accessible through the hinged lid 312 which can be secured tightly with two accompanying latches 316, shown in Fig. 11(a). As shown in Fig. 11(c), the microscope slide is made removable with a microscope slide clip 318 and raised guides. A drain hole 320 in the lower duct allows for drainage of any pooled excess condensation.

**Translating Gap Head**

Complete coverage of the microscope slide with reagents, and nearly constant interaction between these reagents and the tissue sample, are required for the cell staining process. Cell staining tests require the removal of the reagents after the test is completed. These reagents need to be removed and replaced with different reagents during a multi-step cell staining procedure. The spreading, mixing, and removal of reagents are achieved with the use of a translating gap head 400 as shown in Fig. 13(a)-Fig. 15.

The translating gap head works on the principle of capillary attraction. After reagents are dispensed on to the microscope slide 402, the translating gap head makes contact with the reagent while maintaining a physical gap of about 0.003 inch between the surface of the translating gap head 400 and the top surface of the microscope slide, as shown in Fig. 14(a). The translating gap head then moves laterally back and forth across the slide (Fig. 15), dragging along with it the reagent through capillary attraction, which effectively covers the entire slide with reagent. Using this method, reagent volumes as low as 25 micro liters are sufficient for complete coverage of the microscope slide.

It has been found that promoting even greater mixing of the liquids on the slide, which may be reagents or other liquids, while the translating head is translated along its long axis, would be advantageous. Better mixing can allow use of less reagent, which saves on costs and in some circumstances, processing time.

One or more mixing extensions 404 or projections, as shown, e.g., in Figs. 13(b), 13(f) and 13(g) can be added to the bottom surface of the translating head. The mixing extension can be positioned even closer to the slide (e.g., by a gap G2 of about 80 microns, Fig. 14(c)) than the rest of the translating head (i.e., by a gap G1 of about 400 microns, Fig. 14(b)). The mixing extension is typically narrower than
the width of the translating head. In one embodiment, the mixing extension is about 25% of the width of the translating head. When the head is caused to translate, liquid L on the slide at a level above the first gap will be pushed by the approaching mixing extension, causing some of it to deflect laterally. The deflected liquid seeks a path of least resistance and tends to flow laterally along the advancing head until it encounters a part of its leading edge that is separated from the slide by a greater gap. Thus, the mixing extension promotes lateral movement and mixing of the liquid(s) on the slide even when the primary motion of the head is translation in the axial direction.

Small holes 406 in the translating gap head's contact surface allow for the removal of the reagents from the microscope slide by means of vacuum aspiration. When a cell staining test is complete, suction is applied to the translating gap head as it moves laterally back and forth across the microscope slide to effectively remove all reagents.

A translating gap head according to another embodiment is shown in Figs. 16(a)-16(g), 17(a)-17(c), and 18(a)-18(f). In addition to providing suction or vacuum aspiration for removal of liquid from the slide, these embodiments include passageways for providing reagents and/or rinse buffers from the translating gap head.

Referring to Fig. 16(a), which is a perspective view, a head 800 includes a cap 820 that fits over a base 822. Fig. 16(b) is a top plan view of the head 800 showing three rinse inlet ports 824. In the head 800, in addition to removal of reagent by vacuum aspiration, there is a capability to supply rinse liquid under pressure to allow for controllably rinsing the slide, such as before each step in a staining process. Fig. 16(c) is an elevation view in section of the head 800 that shows the rinse inlet ports 824 in the cap 820 and passages 806 in the base 822 through which vacuum is applied.

Fig. 16(d) is a section view in elevation showing passageways 826 for conveying the rinse liquid to the slide. In the illustrated embodiment, the passageways are configured in the spaces between the cap 820 and the base 822.
Fig. 16(e) is a front elevation view of a magnified portion of the head 800. Specifically, Fig. 16(e) shows a lateral guide surface 830, a rail surface 812 and a lower surface of the head 805 spaced about 50 microns above the rail surface 812.

Fig. 16(f) is a perspective view similar to Fig. 16(a), except with a portion of the head 800 cut away to show the arrangement of the passages 806, the rinse liquid passageways 826 and a vacuum port 810. Fig. 16(g) is another perspective cutaway view of the head 800.

Fig. 17(a) is a bottom plan view of the body 822 showing the array of vacuum inlets and the rail surfaces 812. Fig. 17(b) is a front elevation view of the body 822. Fig. 17(c) is an elevation view in section showing the intersection between the passages 806 and the vacuum port 810.

Fig. 18(a) is a perspective view of the cap 820. Fig. 18(b) is a plan view of the bottom side of the cap showing the rinse inlet ports 824, as well as rinse bleed slots 830 and distribution channels 832 that lead from the rinse inlet ports. Fig. 18(c) is sectioned side elevation view. Fig. 18(d) is a sectioned front elevation view showing the rinse inlet ports 824 and one of the bleed slots 830. The bleed slot 830 for center rinse inlet port 824 is shown in a magnified elevation view in Fig. 18(e). Fig. 18(f) is a perspective view showing an underside of the cap 820.

20 Translating Gap Assembly

As shown in Fig. 19, the lateral back and forth motion of the translating gap head is achieved through a screw-driven linear slide 550 which is powered by a two-phase stepper motor 502. The screw-driven linear guide was custom machined to fit the compact space of the interior cell staining chamber 302. The vacuum aspiration required for the removal of the reagents was achieved through an electrical air pump (compressor) 518 that contains both an outlet for positive pressure as well as an inlet for suction. Tubing is connected to a threaded barb valve in the back of the translating gap head mount. This tubing then exits the chamber 302 where it is passes through a solenoid air valve 608 which controls the suction. The tubing is then connected to a bottle 520 that allows for the collection of exhausted reagents. The bottle also contains another port where tubing connects the bottle to the suction port of the air pump 522. An interconnecting air actuator 504 between the
translating gap head 400 and the screw-driven linear slide provides up and down motion to the translating gap head, as shown in Figure 20. This actuator is controlled by an air valve 524 that is attached to the air pump.

The actuator serves two purposes. First, it allows for the removal of the microscope slide from the chamber by lifting the translating gap head vertically away from the microscope slide. The linear guide then moves the translating gap head horizontally, allowing enough room for microscope slide removal. The second function the actuator 504 performs is keeping the tight tolerance of 0.003 of an inch between the contact surface of the translating gap head and the top surface of the microscope slide (Fig. 21). The actuator 504 provides constant pressure to the rail surfaces 412 of the translating gap head which are in contact with the rail surface 414 of the inner chamber (Fig. 14(a)). The rail surface of the inner chamber is the same surface the microscope slide rests on. The translating gap head is designed so that as long as there is contact between its rail surfaces and the inner chamber, the proper gap tolerance between the head and microscope slide is maintained.

**Heating with Steam & Air Mixture**

In order to obtain the proper interaction between antibodies and antigens during the cell staining process, heating is required. Depending on the specific test being performed and the specific reagents used, a particular target temperature between a range of 35-85 °C is required. It has been researched and suggested that steam could be used as a heating element due to the fact that it will prevent evaporation of reagents from the microscope slide during the heating phase. Steam is also a much faster method of heating than through conduction.

Inputting steam at 100°C into a cell staining chamber to reach a target temperature can be achieved by a feedback control system. This method of temperature control often leads to temperature overshooting and is time consuming when changing from one temperature to another. In order to increase the amount of temperature control for the cell staining process, it is proposed that steam is mixed with air to reach a target temperature before entering the cell staining chamber, thus effectively adding an element of control to the thermal process. It is important to note that a feedback controller is not necessary with the application of mixing steam
and air. For example, the steam air mixture at the target temperature can simply be continually pumped into the system to maintain an equilibrium temperature.

With known properties of both steam and air, such as mass, \( m \), specific heat, \( c \), and initial temperatures, different mixing ratios of steam and air allow for a target temperature to be achieved. Equation 6 represents the target temperature as a function of the mass ratio of both steam and air.

Mixing steam with air for the heating phase of the cell staining process increases the amount of control and fine tuning when achieving a set point temperature with tight tolerances. The advantage of adding this element of control to the thermal system allows for advances in efficiency of the cell staining process. The only concern with using pre-determined ratios for each set-point temperature is the rate at which they are achieved. Equilibrium will eventually be achieved, but this may take longer than the 3 minutes or less goal.

\[
T_{\text{final}} = \frac{m_1 c_1 T_1 + m_2 c_2 T_2}{C_{\text{final}}(m_1 + m_2)}
\]

**Heating Assembly**

The heating element of the cell staining chamber includes a system that mixes steam with air which then enters the chamber to achieve a target temperature. The system first feeds small amounts of water from a pressurized bottle 630 by means of a spider valve 602 into a flash boiler 600. The spider valve is a water valve that is capable of delivering volumes of water as small as a few picoliters. The flash boiler 600 is a custom made resistive heating devise that instantaneously turns water into steam, as shown in Figs. 22(a)-22(c). The flash boiler is made from a single piece of aluminum that has a cylindrical channel (bore) through its center with an inlet for water and an exit for steam. The flash boiler 600 is heated by two Hi-Temp Cartridge heaters that are inserted into the front face of the aluminum block on both sides of the steam channel. These cartridge heaters are controlled by an electric temperature controller which has a temperature gage and a thermocouple that is also inserted into the front face of the aluminum block.

The flash boiler is insulated with high temperature fiberglass board. After the input water changes phase into steam, it then travels through tubing where it then
enters a "wye" mixing valve 604, shown in Figure 23. Air of a known temperature and quantity also enters the "wye" mixing valve through a solenoid air valve 606 which is feed by the air pump. The steam/air mixture, now at the target temperature, enters the chamber 302. The mixture travels through the chamber and heats all internal elements as well as the microscope slide containing the sample to be tested while affectively controlling the evaporation of the reagents from the microscope slide.

**System Controls**

Arduino is a tool for the design and development of embedded computer systems, consisting of a simple open hardware design for a single-board microcontroller, with embedded I/O support and a standard programming language. An Arduino is programmed using the *Wiring* language, which is essentially C++ with a few simplifications. An Arduino Duemilanova mainboard microcontroller 700 was used for this project to automate the entire system. It was chosen for reasons of low cost, small component sizes, simple open source coding language, and ease of operation.

The Arduino, which acts as the "brain" of the system, is programmed to control other components that handle specific tasks. For this project, five mechanical relays are used to control the five valves (four air valves and one water valve). A relay is an electrically operated switch that uses an electromagnet to operate the switching mechanism. It is placed in the circuit of a component that is being powered by a separate power source, and allows current to flow when it is closed. The Arduino is programmed to send a voltage output to the relay, which triggers the switch to open or close. This is the mechanism by which all five valves in the system are accurately controlled.

The stepper motor driver 503 is another component that is controlled by the Arduino, and is used for precise control over the 4-wire bi-polar stepper motor 502. The motor 502 requires 24V from a power supply, and is connected to the driver 503. The driver 503 is then connected to the Arduino output. The Arduino is programmed to output SV to the driver to make the motor rotate either clockwise or counter-clockwise in a set amount of rotations per second. This is the mechanism
moves the translating gap head assembly back and forth along the linear screw guide 500.

System Integration

In terms of controls, the system is divided into two parts; heating the chamber and spreading/removing reagents. Each part is being controlled by its own Arduino microcontroller, so that two separate programs can be running simultaneously. The first Arduino chip 700 that controls the heating aspect has outputs to two valves, one for air (606) and one for steam (602), and an LCD display 702. The functional requirements for heating state that the chamber must be heated to any temperature between 35-85°C (±1°C) and remain steady. It must also reach this set-point temperature in three minutes or less. With steam heating as a constraint, it was decided that an air-steam combination would be the best way to maintain temperatures below 100°C. This is why both an air and water valve are necessary for the heating aspect of the design. To meet the timing requirement, but avoid overshooting the temperature, a two stage air-steam combination program with feedback was implemented. Air and steam combinations are made by controlling the pulsing rate of the air valve and water valve (that feeds into the flash boiler to produce steam) individually. The combinations were made to be able to heat quickly at first (mostly steam), and then slower as the chamber temperature approaches the set-point temperature. The exact valve pulsing rates to achieve these optimal combinations were determined during testing. When the chamber finally reaches the desired temperature, no heat is added. If the chamber goes above the desired temperature, air is used to cool it down. A temperature sensor 706 mounted inside the chamber is the mechanism used for feedback. The code is written with the set-point temperature as a user input. It reads in the temperature from the sensor, calculates how far off it is from the set-point, determines which range the system currently is in, and activates the appropriate air-steam combination. The LCD screen 702 displays the real time chamber temperature.

The second Arduino chip 710 (Fig. 24(b)) for the reagent spreading and removal aspect of the design, has outputs that control two air actuator valves, one air valve for vacuum suction, and the driver 503 that runs the stepper motor 502. The
air actuator works by two air input ports. Air into one port raises the translating gap head. Air into the second port lowers it. Through voltage output to the mechanical relays, which open and close the air valves, the second Arduino chip 710 is programmed to lower the translating gap head into position so it can spread, mix and vacuum the reagents from the slide. It then raises the translating gap head so that it can pass above the slide clamp and return to its inactive position. The air valve for suction stops the flow of air being sucked into the pump. The second Arduino chip 710 opens the valve when the translating gap head is moving back and forth across the slide so that it can suck the reagents up at the end of the process. The air suction tube runs from the translating gap head through a collecting bottle for the reagents, into the valve, and then to the pump inlet. The 2-phase motor 502 is the last component controlled by the second Arduino chip 710, through intermediary action of the stepper motor driver.

Both Arduino chips will output to control a panel of LEDs that are used to inform the viewer which stage the system is in. Also, an LED will be used to indicate when the chamber has reached the set-point temperature within ±1°C.

Figs. 24(a) and 24(b) are circuit diagrams, and Fig. 25 is a systems control and integration diagram.

20 System Build

High density Styrofoam Insulation was cut with a hot wire CNC foam cutter and was then inserted between the inner and outer shells of the adiabatic chamber. Silicon and weather stripping was then applied to all seams of the inner and outer shells, creating an air tight seal. The translating gap assembly was then placed in position and secured to the inner chamber wall with screws. This chamber assembly was then attached with screws to a custom fabricated sheet metal mount. This mount was then attached to a 2'X2'X1.5" hollow steel platform. All components such as air valves, air compressor, water bottles, pressure gages, etc., were secured to the steel platform with screws. Tubing and wires were then attached to all corresponding components and secured with zip ties.
Verification and Validation

As indicated, the chamber should be heated to any desired set-point temperature between 35-85°C in less than three minutes and maintained within +1°C.

With steam heating as a constraint, it was necessary to find a way to maintain temperatures below 100°C. The chosen method was to use air and steam mixtures, which depending upon the ratio of air to steam, would accomplish this. According to one approach, for each temperature between 35-85°C, the control system would be programmed with a pre-determined combination of air and steam.

This type of system would require extremely precise valve control, and given the time limitations, would not be feasible. Another approach is to use a feedback control system. This would enable one to simply input any set-point temperature, and the system could achieve it using more than one air-steam combination. The combinations can be created by adjusting the pulsing rates of the air and water valves. Through testing various combinations, it can be determined how fast and what final temperatures the chamber can reach. Based on these preliminary test results, one combination will be chosen for its ability to heat rapidly. This will serve as the stage 1 combination that is called on get the chamber temperature close to the set-point. Then, based on the temperatures that are achievable using the various combinations, a few stage 2 combinations will be chosen. A code can be written to create two temperature ranges below the user-defined input temperature. The first range covers all temperatures up to 10 degrees under the set-point. For this stage, the fast heating combination (Combo 1) is used. In the range from 10 degrees below set-point up to set-point temperature (stage 2), a second combination would be used.

Combination 1 would be constant for any input temperature. The stage 2 combination would vary depending on the set-point temperature. This type of 2-stage feedback system provides the ability to meet two functional requirements. It combines the benefits of fast heating to meet time constraints and slower heating to reach and maintain set-point temperature without overshoot. When the temperature is finally met, the heating becomes inactive, and the insulation holds the temperature steady. If there is slight overshoot, air is blown into the chamber to cool it down.
Any slight drop below set-point and the Arduino activates stage 2 heating to slowly bring it back to set-point.

By adjusting the pulsing rates of the air and steam valves, various combinations can be made and plotted on a Temperature vs. Time graph. This data will be used to choose the best combinations for the 2-stage control system. After programming the Arduino microcontroller with these combinations, it is necessary to test multiple temperatures within the 35-85°C range. The final plot will contain results of achieving set-points for the two extremes (35°C, 85°C) and the midpoint temperature (60°C).

**EXAMPLES**

The tests to determine which air-steam combinations will be used in the feedback program provided extremely useful results. Temperatures above 85°C, as low as 35°C, and many in between were met well within the three minute time constraint. When the air and steam valves pulsed at the same rate, a 1 second adjustment in the delay time between pulses resulted in a nearly consistent 10°C change in final temperatures reached. For example, combination 8 had both the air and steam valves pulsing every 4.5 seconds. This combination allowed us to reach 50°C. When the pulse rate was changed to 3.5 seconds, the chamber reached 60°C.

When it was shortened again to 2.5 seconds, the chamber came close to reaching 70°C. This type of linear increase in temperature with changing pulse rates made it easy to figure out how to reach any temperature between the ones that were measured. The fastest pulse rate that was used was 0.5 seconds. This rate heated the chamber to 90°C, but it took about 2 minutes. By keeping the steam valve rate at 0.5 seconds but adjusting the air valve pulse rate, the chamber able to be heated even quicker. Combo 1, 2, and 3 in the results are the combinations that used different air and steam pulse rates. Combination 1 (0.5 second steam rate, 2.3 second air rate) provided the fastest initial increase and highest attainable temperature. Fig. 26(a) is a plot of Temperature vs. Time for the 8 combinations that were tested. Fig. 26(b) contains tabulated temperature observations for the eight combinations. Fig. 26(c) summarizes the air delay values and steam delay values for the respective combinations.
Combination 1 could reach 80°C within 1 minute, so this was chosen as the stage 1 combination in the feedback code. The stage 2 combination is chosen based on its ability to reach the user-defined set-point temperature. For example, combination 7 can reach 60°C within 3 minutes. Combination 8 can only reach 50°C within 3 minutes. Therefore, if it is desired to reach a temperature between 50-60°C, Combination 7 is chosen as the stage 2 combo. Using this logic, a single stage 2 combination was chosen for each of the five temperature ranges (35-50, 50-60, 60-70, 70-80, 80-85) between 35-85°C. After programming the Arduino to run this type of 2-stage feedback control system, it was tested by trying to reach set-points at increments of every five degrees from 35-85°C. The results show that this type of control system worked just as expected. For each input temperature, the code ran stage 1 until 10°C below the set-point temperature, and then switched over to the stage two combination. Each temperature tested gave similar results. In stage 1, the chamber was heating very rapidly. This was visible on the LCD display of the chamber temperature. As soon as the program switched to stage 2, the chamber temperature rose very slowly (about 1°C every 5 seconds) until the set-point was met. Because of the feedback control, the set-point was kept within ±1°C. A graph of the results for the 35, 60, and 85°C tests are shown in Fig. 27(a), with the supporting data provided in Fig. 27(b). These three test results show that the system can successfully meet and maintain the lowest, highest and midpoint temperatures in the desired range.

The results from this test verify that the system is capable of achieving any set-point temperature in the range of 35-85°C in less than 2 minutes. It is also capable of maintaining that temperature within ±1°C.

**Functional Requirement:** Maintain temperature uniformity across the entire slide within +0.5°C.

In order to verify that the microscope slide is being uniformly heated, a test slide 402 was divided up into a grid for the placement of 6 temperature sensors. Due to limited number of analog inputs on the Arduino, six was the maximum amount of sensors that could be read at once. For that reason, it was decided that two rows of three sensors (Fig. 28(a)) that run the length of the slide would provide
enough data to determine how the uniformity was being affected. Based on the chamber design of a flow guiding duct beneath the slide, it was expected that the slide would heat at the end nearest the steam entrance first, and then become more uniform as the chamber reaches equilibrium.

Six temperature sensors were attached to the pre-determined locations on the slide. The chamber was heated from room temperature to above 80°C. All of the sensors values were displayed on the LCD and recorded every 30 seconds for four and a half minutes. This length of time was chosen to ensure that the chamber could come to a natural equilibrium, since no feedback or control system was being used to stop it from heating. The test was performed twice so that the results of trial 1 could be compared with those of trial 2 (Fig. 28(b)).

**Results and Discussion:**

Both tests produced similar results, but the first showed slightly better overall uniformity. Comparing the data from both tests, the original hypothesis was verified. The two sensors on the edge of the slide nearest the air-steam entrance duct were hotter than the two sensors in the middle. The two middle sensors were hotter than the two sensors at the far edges, thus showing that the direction and location of steam flow does affect the slide uniformity. Despite this initial variation in temperature along the length of the slide, data showed that after about 2 minutes, the slide became increasingly more uniform. By the end of 270 seconds (4.5 minutes), it was possible to achieve uniformity within 1.25°C (Fig 28(b)).

Despite being very close, these results do not satisfy the ±0.5°C allowance set forth as a goal. The 1.25°C was determined by subtracting the minimum from the maximum value of the six sensor readings at the end of trial 1 (the better of the two tests) when the slide was at its best uniformity. Considering that ±0.5°C is the same thing as 1°C between the max and min value, this system is only 0.2°C from being capable of achieving uniformity.

**Functional Requirement:** Spread reagents to cover the entire slide before testing, keep them mixing during a staining test, and completely remove them at the end.
Using a translating gap head, an assembly was built that connects the head to a linear guide. The 2-phase motor provides the back and forth motion that moves the head along the slide and spreads the reagents. The same motion is used to remove the reagents through vacuum holes in the translating gap head.

Methods:

Before testing could be done, it was necessary to program the Arduino to control the forward and reverse motions of the 2-phase motor. Once the distances (forward and backward) were defined, the test was run to determine the optimal speed for spreading and vacuuming. These were done by running the cycle several times using different speeds and visually analyzing (by spreading colored dye) which speed works best.

Results and Discussion:

After applying 100 microliters of food coloring (to represent a staining reagent) to the center of the slide, the system was capable of spreading the dye to achieve full coverage of the slide. Testing showed that within a certain range, varying the speed of the gap head did not have a significant effect on spreading. The speeds did however have a greater effect on the vacuuming/removing reagents phase. These tests showed that the slower the speed, the more effective the gap head is at removing reagents. Based on these tests, the program was written to use the slowest speed that maintained full spreading and mixing.

The system was able to spread the reagents to achieve full slide coverage. It kept the head moving during the entire test to accommodate the mixing requirement, and then successfully vacuumed all of the reagents from the surface to conclude the test.

Validation:

Shortcomings of the prior approaches were recognized and addressed in the process of developing the present apparatus and methods. It was recognized that excess buffer volume diluted the reaction mixture. Using a less dilute reaction mixture solution was considered as possibly reducing incubation times in a staining
procedure and thereby allowing results to be delivered more quickly. Vortex mixers sometimes mixed the reagents unevenly and created dry spots on the slide. It was considered that these effects might be mitigated by mixing the reagents on the slide in a different way. Heating the incubating mixture conductively by placing an electrical heating plate in contact with the slide is a workable approach, but it was observed that heat is sometimes distributed unevenly and the process can take considerable time. Steam was proposed as an alternative for heating the incubating mixture based on the versatility and efficiency of steam heating. It was recognized that overheating the sample and reagents caused excessive evaporation of the reagents from the sample, thus drying the sample and rendering it less suitable for testing. It was also recognized that although oil can be layered on top of reagents to reduce evaporation, it is now considered a pollutant and must be treated as bio-waste, which adds costs.

With the exception of perfect slide temperature uniformity, the system met the goals because it:

- Achieves a user-defined steady set-point temperature (within ±1°C)
- Changes temperatures rapidly (within 3 minutes)
- Spreads reagents to cover entire slide
- Keeps the reagents mixing

- Removes reagents from the slide through vacuum aspirations
- Reduces evaporation of the reagents due to 100% steam humidity
- Performs all tasks without manual control (fully automated)

The cell staining system, as optimized by a steam-air mixture for heating and translating gap for reagent spreading, demonstrates that this design is a viable solution for the next generation cell staining system.

The integration of translating gap proved to be an effective way to spread, mix, and remove reagents. With this method, there is a significant reduction in reagent volumes required to cover the entire surface of the slide. The novel idea of steam heating proved effective in decreasing test times by speeding up the heating process. In addition, the 100% humidity created by steam eliminates reagent evaporation at high temperatures. Without the threat of evaporation, there is no
longer a need to cover the tissue sample and reagent with a protective oil layer. By eliminating the need for oil, we reduce the cost of waste treatment processes associated with bio wastes.

Through the use of air-steam combinations in a 2-stage automated feedback system, all temperatures within the desired range of 35-85°C were met in less than two minutes, and maintained within ±1°C. The air-steam combinations were created by altering the pulse rates of the air and steam valves. Our system uses a very simplified and almost linear arrangement of pulse rates that determine combinations. Since the achievable set-point temperatures were extremely sensitive to changes in valve timing, a fine tuning of the ratios and an increase from a 2-stage to 3 or 4-stage feedback system could easily improve the heating times and preservation of set-point temperatures.

The uniformity test results showed that the temperature distribution across the width of the slide had very little variation. The temperature uniformity down the length the slide was shown to be within 1.25°C at chamber equilibrium, but varied by as much as 20.44°C during the transition phase. A repeatable trend showed that the temperature sensors nearest the steam-air input reached values much higher than those placed farther down the slide. This result is due to the slide placement in close proximity to the steam-air input. Here we see the undesired effects of vertical heating as discussed in the preliminary design section. Turbulence within the flow is creating stagnant concentrations of hot steam near the inlet. This non-uniform temperature distribution could be improved simply by placing the slide farther from the steam-air input. The location needs to be in the region where the flow is laminar through the duct.

As an alternative to the translating gap approach, a cap shaped to contact and spread liquid on the slide can be used instead of the translating head shown in Figs. 8(a) and 15. Referring to Fig. 8(b), a cap 201 may have a curved surface dimensioned to have a length approximately as long as the working length of the slide 60. In this way, the cap 201 can be caused to rock back and forth to spread liquid reagent 40 on the slide 60, without requiring the cap 201 to translate back and forth. The rocking action of the cap 201 shifts the meniscus formed in the liquid reagent 40 on the slide 60 back and forth. In the illustrated embodiment, there are
outer rails 202 provided at each end and having a height set to establish a gap between the cap 201 and the slide 60, or between the cap 201 and the liquid reagent 40 on the slide 60, as desired. In the illustrated embodiment, the rails 202 are each about 50 microns in height. The slide 60 may have a bar code identifier, such as a bar code label 100 as shown in Fig. 8(b).

A production apparatus handling multiple slides, such as the assignee's BENCHMARK® automated slide preparation system, can employ the translating gap approach or the rocking cap approach, among others, to accomplish mixing, in conjunction with the air quenched steam heating described above. To employ steam heating effectively, a reaction chamber is defined by at least the working area of the slide surface and structural elements positioned to the sides of and above the slide.

There may also be another structural element positioned below the working surface of the slide. The reaction chamber is constructed similar to the insulated chamber 302 to minimize loss of heat from within the space where the steam is being applied.

In view of the many possible embodiments to which the disclosed principles may be applied, it should be recognized that the illustrated embodiments are only preferred examples and should not be taken as limiting the scope of protection. Rather, the scope of protection is defined by the following claims. We therefore claim all that comes within the scope and spirit of these claims.
We claim:

1. An automated cell staining apparatus, comprising:
   a chamber dimensioned to enclose a microscope slide having a sample thereon;
   a steam heat source capable of heating the chamber, microscope slide and sample with steam to a desired temperature; and
   an air source capable of cooling the steam from the steam heat source with air for controllably reaching the desired temperature.

2. The apparatus of claim 1, wherein the steam heat source is capable of heating the microscope slide and sample to a desired temperature between 35°C to 85°C.

3. The apparatus of claim 1, wherein the apparatus is capable of maintaining the desired temperature within 1°C over a selected duration of testing.

4. The apparatus of claim 1, wherein the apparatus is capable of maintaining different points across the microscope slide within 1.25°C of the desired temperature.

5. The apparatus of claim 1, wherein the steam heat source is capable of heating the sample to the desired temperature within about 3 minutes.

6. The apparatus of claim 1, wherein the steam heat source is capable of heating the sample to the desired temperature within about 2 minutes.

7. The apparatus of claim 1, wherein the steam heat source includes a flash boiler connected to a source of water, the flash boiler being capable of heating water to steam by resistive heating.
8. The apparatus of claim 7, wherein the steam heat source includes a spider valve positioned upstream from the flash boiler and downstream from the source of water, the spider valve being capable of feeding small volumes of water to the flash boiler.

9. The apparatus of claim 1, further comprising a controller programmed to heat the slide quickly using a first stage combination of steam and air to a temperature close to but less than the desired temperature followed by a second stage combination of steam and air to heat the slide to the desired temperature with minimal overshooting.

10. The apparatus of claim 1, wherein the slide is positioned substantially level and the source of steam heat is configured to direct steam substantially parallel to a lower surface of the slide.

11. The apparatus of claim 1, wherein the steam is directed in a substantially laminar flow and heats the slide and sample through condensation heating.

12. The apparatus of claim 1, further comprising a generally horizontal duct positioned beneath the microscope slide, and wherein the duct is capable of guiding steam and air entering the chamber in a direction along a lower surface of the slide until the steam and air exit the duct and are caused to flow over an upper surface of the slide.

13. An automated cell staining apparatus, comprising:

an insulated chamber dimensioned to enclose a microscope slide having a sample thereon, the chamber comprising a steam and air inlet in a lower surface, a duct leading upwardly from the steam and air inlet and having a horizontal portion along a lower surface of the microscope slide, a vent in an upper surface of the chamber, and a temperature sensor capable of sensing the temperature in the chamber;
a steam heat source capable of supplying steam to heat the chamber, the microscope slide and the sample to desired temperatures by condensation heating, the steam heat source comprising a flash boiler powered by cartridge heaters and a controllable steam valve;

an air source capable of generating air at a temperature cooler than the steam, the air source comprising an air pump and a controllable air valve;

a wye valve through which steam from the flash boiler and the controllable steam valve, and air from the air pump and the controllable air valve, are combined and fed to the air and steam inlet of the chamber, the resulting mixture having a temperature less than a temperature of the steam; and

a controller connected to the temperature sensor, the controllable steam valve and the controllable air valve, wherein the controller is programmed to cause the chamber to reach a desired set point temperature by actuating the controllable steam valve to supply more steam if a current temperature sensed by the temperature sensor is below the desired temperature and by actuating the controllable air valve to supply more air if the current temperature sensed by the temperature sensor is above the desired temperature.

14. The apparatus of claim 13, wherein the steam heat source is capable of heating the sample to the desired temperature within about 3 minutes.

15. The apparatus of claim 13, wherein the apparatus is capable of maintaining the desired temperature within 1°C over a selected duration of testing.
<table>
<thead>
<tr>
<th>Design for Steam Heating</th>
<th>Customer Importance Multiplier</th>
<th>Current System: Benchmark ULTRA</th>
<th>Weighted Value</th>
<th>Design 1: Vertical Heating</th>
<th>Weighted Value</th>
<th>Design 2: Horizontal Flow</th>
<th>Weighted Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control temperature on the slide</td>
<td>5</td>
<td>3</td>
<td>15</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Control evaporation</td>
<td>4</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Maintain temperature uniformity</td>
<td>4</td>
<td>3</td>
<td>12</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>Change temperature rapidly</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>9</td>
<td>27</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td></td>
<td><strong>75</strong></td>
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<table>
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<th>Design for Spreading</th>
<th>Customer Importance Multiplier</th>
<th>Benchmark ULTRA</th>
<th>Weighted Value</th>
<th>Design 1: HULA</th>
<th>Weighted Value</th>
<th>Design 2: Translating Gap</th>
<th>Weighted Value</th>
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</thead>
<tbody>
<tr>
<td>Decrease amount of reagent used</td>
<td>4</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>36</td>
</tr>
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<td>Spread Reagent</td>
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<td>3</td>
<td>12</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>36</td>
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<tr>
<td>Improve reagent-tissue interactions</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>9</td>
<td>27</td>
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<td>Remove reagent thoroughly</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>18</td>
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<tr>
<td><strong>Total</strong></td>
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<td><strong>57</strong></td>
<td></td>
<td><strong>117</strong></td>
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</table>

**Weights:**
1 = does not meet criteria
3 = meets criteria
9 = exceeds criteria

FIG. 9
FIG. 26(a)
<table>
<thead>
<tr>
<th>Combination</th>
<th>Air delay (sec)</th>
<th>Steam delay (sec)</th>
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</thead>
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<tr>
<td>Combination 1</td>
<td>2.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Combination 2</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Combination 3</td>
<td>NO AIR</td>
<td>0.5</td>
</tr>
<tr>
<td>Combination 4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Combination 5</td>
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<td>Combination 6</td>
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<td>Combination 8</td>
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</tr>
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</table>

FIG. 26(c)
Achieving Set-Point Temperatures

FIG. 27(a)
<table>
<thead>
<tr>
<th>Time(s)</th>
<th>Air = Steam = 4.5 (Temp (Celsius))</th>
<th>Air = Steam = 1.5 (Temp (Celsius))</th>
<th>Air = Steam = 0.2 (Temp (Celsius))</th>
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</thead>
<tbody>
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<td>0</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
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<td>5</td>
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<td>27.12</td>
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<td>27.12</td>
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<td>27.62</td>
<td>60.62</td>
<td>55.93</td>
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<td>28.62</td>
<td>59.8</td>
<td>62</td>
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<td>30</td>
<td>30.18</td>
<td>58.2</td>
<td>68.43</td>
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<td>35</td>
<td>31.93</td>
<td>58.8</td>
<td>72.62</td>
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<tr>
<td>40</td>
<td>35.58</td>
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<td>45</td>
<td>35.18</td>
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<tr>
<td>60</td>
<td>35.5</td>
<td>60.3</td>
<td>79.25</td>
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<td>65</td>
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<td>59.5</td>
<td>80</td>
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<td>35.12</td>
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FIG. 27(b)
Temperature Variation Across Slide

Max-Min (Celsius) vs. Time (seconds)

Trial 1
Trial 2

FIG. 28(b)
**INTERNATIONAL SEARCH REPORT**

**International application No.**

PCT/US 1/34824

**A. CLASSIFICATION OF SUBJECT MATTER**

<table>
<thead>
<tr>
<th>IPC(8) -</th>
<th>G01N 1/00 (201.1.01)</th>
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<td>USPC -</td>
<td>436/46; 422/63</td>
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</table>

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

- IPC(8) - G01N 1/00 (201.1.01)
- USPC - 436/46; 422/63

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

- Non-patent Literature; Patents (key word limited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

- PubWEST (PGPB, USPT, EPAB, JPAB); Google (Google Scholar, Google Patents)
- Search Terms Used: cell staining, slide staining, slide stainer, apparatus, chamber, staining, immunostaining, immunohistochemical, in situ hybridization, histochemical staining, automated, automatic, automatically, microscope, slide, sample, steam, steaming, steamer

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
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<tbody>
<tr>
<td>X</td>
<td>US 2010/0068757 A1 (ANGROS) 18 March 2010 (18.03.2010) para [0006], [0064]-[0065], [0085], [0096], [0121], [0124], [0141]-[0142], [0188], [0279]-[0285], [0287]-[0288], [0346]; fig 13, 26; elm 9</td>
<td>1-2, 3-15</td>
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<td>Y</td>
<td>US 5,746,009 A (BRUGGER) 05 May 1998 (05.05.1998) col 2, ln 25-34; col 3, ln 3-25, 35-40, 50-61; fig 1; elm 1; ab</td>
<td>3-4, 9, 12-15</td>
</tr>
<tr>
<td>Y</td>
<td>US 5,722,566 A (GLYNN) 03 March 1998 (03.03.1998) col 2, ln 28-41; elm 7</td>
<td>8</td>
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</table>

* Further documents are listed in the continuation of Box C.

**Date of the actual completion of the international search**

01 July 2011 (06.07.2011)

**Date of mailing of the international search report**

11 JUL 2011

**Name and mailing address of the ISA/US**

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

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