Published in final edited form as:

AIChE J. 2016 June ; 62(6): 2227–2233. doi:10.1002/aic.15194.

Temperature gradients drive radial fluid flow in Petri dishes and multiwell plates

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Abstract

Liquid in a Petri dish spontaneously circulates in a radial pattern, even when the dish is at rest. These fluid flows have been observed and utilized for biological research, but their origins have not been well-studied. Here we used particle-tracking to measure velocities of radial fluid flows, which are shown to be linked to evaporation. Infrared thermal imaging was used to identify thermal gradients at the air-liquid interface and at the bottom of the dish. Two-color ratiometric fluorescence confocal imaging was used to measure thermal gradients in the vertical direction within the fluid. A finite-element model of the fluid, incorporating the measured temperature profiles, shows that buoyancy forces are sufficient to produce flows consistent with the measured particle velocity results. Such flows may arise in other dish or plate formats, and may impact biological research in positive or negative ways.

Topical Heading and Key Words

Transport phenomena and fluid mechanics: natural convection; thermal imaging; confocal microscopy; particle tracking; virus comet assay; Petri dish; COMSOL; computational fluid dynamics

Introduction

Convective flows of liquid media in Petri dishes and multi-well plates (“wells”) can have a significant effect on the conditions and outcomes of biological experiments. For example, the virus comet assay exploits such spontaneous flows to enhance the spread of virus infections across a monolayer of susceptible cells, producing visible comet-like patterns of cell death¹. In the more conventional plaque assay, placing a gel or high-viscosity solution over the cell monolayer inhibits such flows, limiting infection spread and producing localized circular regions called “plaques” where the cell monolayer has been damaged by cell death, detachment, or other cytopathic effects². Figure 1 illustrates the morphology of infection spread for influenza virus plaques and comets. Small circular plaques are easier to count, providing a basis for measures of infectious virus by the plaque assay. By contrast, comets tend to spread over a much greater area, making the comet assay useful for sensitive

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testing of antiviral drugs^{3,4}. The comet assay is also useful for differentiating between different mechanisms of virus spread, where infection spread by extracellular release of virus particles favors comet production, while infection spread through direct contact between infected and susceptible cells does not⁵. Orientations of comets also reveal flow conditions in the well. For example, each comet's direction is a streamline, and the comet's aspect ratio (length/width) indicates the relative strength of convection over diffusion. In general, the comet assay reveals a strong radial component to flow in six-well plates, as reflected by the starburst pattern of Figure 1b.

The flows that give rise to comets in static wells have not been well understood or characterized. Thermal gradients, possibly from evaporative cooling, have been suggested as the driving force for comet-forming flow in six-well plates^{4,6,7}. This hypothesis is supported by the observation that virus comets formed at lower humidity tend to be more elongated than comets formed at higher humidity levels³. It has been observed that virus comets formed on an inclined plate spread uphill⁶, indicating that convection must dominate over gravity and may arise from temperature gradients. A temperature gradient across a container can give rise to an upward force acting on fluid in one part of a container, and a downward force in a different region, leading to a circulating flow pattern⁸. Circulating flow patterns involving buoyancy-driven flow near an air-liquid interface have been described for other geometries such as heated grooves⁹, inclined plates¹⁰, and capillaries¹¹. In this work, flow velocity measurements, non-invasive thermal imaging, and computational fluid dynamics are used to link temperature gradients in plates and wells with buoyancy-driven flow.

Materials and Methods

Particle tracking experiments

Red fluorescent polystyrene microspheres (Life Technologies, Grand Island, NY) with a diameter of one micron were diluted into a solution (PBS-BSA) composed of PBS (Sigma, St. Louis, MO) with 3 mg/mL bovine serum albumin (Life Technologies). PBS-BSA was used to mimic the physical properties of cell culture media. Two mL of the PBS-BSA solution containing fluorescent microspheres were placed in one 35-mm diameter well of a standard plasma treated polystyrene six-well plate (Fisher Waltham, MA), and incubated at room temperature in a stage-top microscope chamber. Fluorescence images of beads settled at the bottom of the well were acquired using a Nikon TE300 (Nikon, Melville, NY) inverted microscope every 60 seconds for at least 50 minutes at five positions per well. Fluid velocities were calculated by tracking particle movement using the MTrack2 function in ImageJ (Rasband WS. 1997–2012. ImageJ. US National Institutes of Health. Bethesda, MD, USA. <http://imagej.nih.gov/ij/>). Tracks from beads that adhered to the well and stopped moving were ignored, and did not contribute to flow velocity calculations. With a depth of field of 2.88 microns and the center of the focal plane aligned with the center of the one-micron spherical beads at the bottom surface of the well, the in-focus region included the lower 1.94 microns of the fluid in the well. When comparing to simulation, it is assumed that particle-tracking measurements correspond to flow velocities at the midpoint of this range, or approximately one micron from the dish bottom.

Temperature determination by confocal imaging

Temperatures were measured using a two-color ratiometric confocal fluorescence imaging method similar to that described previously¹². The principle behind this method is that the intensity of rhodamine B in solution changes by roughly 2% per °C. For additional robustness against imaging artifacts and fluctuations in laser intensity, the fluorescence of rhodamine B was normalized to a second dye, rhodamine 110, which is relatively insensitive to temperature differences. The B:110 fluorescence intensity ratio is related to temperature through a calibration curve from intensity ratios at known temperatures and depths within the fluid. Confocal imaging allows independent images to be acquired at different depths within a liquid sample, as light is filtered by a pinhole so only in-focus light (originating from very close to the focal plane) will enter the detector.

Confocal imaging setup

In order to create an environment similar in geometry to the comet incubation conditions, but with a bottom surface more amenable to fluorescence and thermal imaging, one 35mm-diameter well was cut from a six-well plate (Corning, Corning, NY) using a razor blade, and the polystyrene bottom was replaced with a microscope cover glass (Fisher, Waltham, MA), attached around the outside edge of the well with silicone adhesive (DAP, Baltimore, MD). The dye solution was composed of 0.1mM rhodamine B (Sigma) and 0.1mM rhodamine 110 (Sigma) in PBS-BSA, for a pH-buffered solution comparable to the infection media from the comet assay in terms of ionic composition, viscosity, and surface tension. Two mL of dye solution was placed in the glass-bottom well, a loosely-fitting cover was placed on the dish, and the covered dish was placed inside a microscope stage-top incubation chamber (Tokai Hit, Shizuoka-ken, Japan). The incubation chamber was placed on the microscope stage with temperature and CO₂ at ambient conditions. The dish was incubated 3.5 hours to allow the system to approach a thermal and flow steady-state condition.

Confocal image acquisition

Following the incubation period, images were taken with a Nikon A1 confocal laser scanning microscope using NIS-Elements software (Nikon). Each scanned point within the fluid was excited with lasers at 488nm and 561nm, to induce fluorescence from the rhodamine 110 and rhodamine B dyes, respectively. Emitted light was passed through a beam splitter to separate the wavelengths from the two dyes, detecting at 500–550nm and 570–620nm using photo multiplier tube detectors. Images were collected in the well along two perpendicular diagonals passing through the center of the dish, forming an “x” as viewed from above. A z-series (collection of images corresponding to different heights in the z-dimension) was acquired at 11 locations evenly spaced across each diagonal. This was done by moving the optics vertically by 25 microns (though the focal plane moves 33 microns: see Z-Step Calibration) between acquisitions. Each z-series spanned the depth of the fluid with several additional frames with lower intensity acquired above and below the fluid boundary so that the location of the fluid boundary could be determined. Motion of the optics in the z-dimension was automated, while stage translations in the horizontal plane were carried out manually, slowly enough to avoid agitating the fluid. Images acquired at two frames/second through a 10× air objective had a size of 256×256 pixels or 1.3×1.3 mm.

The thickness of the optical section was estimated by NIS-Elements software as 16.7 microns.

Temperature calibration for confocal thermal imaging

Rhodamine B image intensity depends on the local temperature as well as the depth, since the emitted light is scattered and absorbed as it passes through the liquid. For the calibration, a type T thermocouple probe was submerged in 3mL of dye solution in the glass-bottom well, and connected to a data acquisition box (Cole-Parmer, Vernon Hills, IL). The fluid was heated to various temperatures using the temperature controller for the stage-top chamber, with a live temperature trace recorded by the thermocouple probe and TracerDAQ software (Cole-Parmer). After the temperature readings stabilized at each condition, the fluid was mixed thoroughly and a z-series was acquired within a few millimeters of the temperature probe at both 488nm and 561nm excitation wavelengths. This was done for a range of temperatures between 24°C and 30°C. These images were used to establish a relationship between temperature, image intensity, and z.

Step size calibration for confocal imaging

For z-series acquisition, the z-step size was set to 25 microns, meaning the optics moved 25 microns vertically. Because the liquid has a refractive index greater than that of air, the focal plane travels further than the physical movement of the optics. The scaling factor between distance traveled by the focal plane and distance traveled by the optics is equal to the ratio of the refractive indices of the liquid and the air (12), or approximately 1.33. This ratio was verified by comparing measured thickness with the vertical travel distance of the microscope optics scanning from the bottom to the top of dye-permeable polydimethylsiloxane (PDMS; prepared using Sylgard 184 kit, Dow-Corning) slabs soaked in rhodamine B dye solution.

Confocal Image analysis

Median grayscale intensity at 488nm and 561nm wavelengths was calculated using the “Multi measure” function in ImageJ. For each z-series, the location of the z=0 plane (the glass-liquid interface) was determined as the plane corresponding to a rhodamine 110 image intensity halfway between the baseline intensity (where the depth of field is below the fluid) and the maximum intensity (where the entire depth of field is within the fluid). The upper surface representing the air-liquid interface was determined as the z-location corresponding to the midpoint in intensity between the fluorescence of the dye solution and the background intensity outside the liquid layer. For each image acquired within the liquid, the image intensity and z position were used to calculate the liquid temperature by comparison to the calibration data.

Because emitted fluorescence from the dye is scattered or absorbed as it passes through the fluid on the way to the detector, the absolute intensity necessarily decreases with distance from the bottom of the well. Above 0.8mm from the lower surface, the signal-to-noise ratio declines and eventually noise dominates. Within 0.8mm from the lower surface of the well the confocal-based thermal imaging method as applied in this study was highly sensitive for detecting relative temperature differences with varying depth if the vertical distance between focal planes was precisely defined, as in a single z-scan from an automated microscope.

Infrared thermography

A thermal imaging camera (A320, FLIR, Boston, MA) was attached to a stand and placed in an incubator at 37°C until the camera and stand reached thermal equilibrium. To measure temperatures at the air-liquid interface, an uncovered 35-mm well containing 2mL of PBS-BSA was placed directly underneath the camera, with the camera pointed downward, and incubated for an additional 20 minutes or longer to reach thermal steady-state before acquiring thermal images. To measure temperatures from underneath, a 35mm-diameter well with a bottom surface of microscope cover glass containing 2mL of fluid, was held above the camera by a non-conducting polymer support. The camera was pointed upward toward the dish bottom. Thermal images of the glass in contact with the bottom of the fluid layer were acquired after at least 20 minutes of thermal equilibration. Images of the air-liquid interface were of necessity acquired with the lid removed, while images of the bottom surface were acquired with the lid on, as in typical use conditions. Images were processed using ThermoCAM Researcher (FLIR) and MATLAB software to extract radial temperature profiles. An emissivity value of 0.97 was used for liquid water and glass. In each case, only the relative profiles (obtained by subtracting the lowest value from the entire profile) were used as quantitative simulation inputs. The A320 camera has a resolution of 320×240 pixels and a thermal sensitivity (noise equivalent temperature difference) of 50mK.

Finite element laminar flow and heat transfer model

A fluid layer in a 35-mm well was simulated in COMSOL Multiphysics using the laminar flow and conduction/convection physics modules in a two-dimensional geometry with axial symmetry. For simplicity, thermal and flow effects arising from uneven heating or air circulation were neglected. The side wall and axial symmetry boundary representing the center of the well were both vertical, perpendicular to the horizontal lower boundary. The lower boundary was 17.5mm long, representing one half of a standard 35mm well, and the upper boundary was defined by the meniscus, determined by confocal imaging. The meniscus geometry was drawn with high resolution as a spline in AutoCAD (Autodesk, San Rafael, CA), and the curve was exported into COMSOL. The fluid geometry was filled with an unstructured mesh of 23,369 elements in COMSOL.

The steady-state incompressible Navier-Stokes and continuity equations (Equations 1 and 2) were used to describe fluid motion, including the Boussinesq approximation for the temperature dependent body-force term on the right side of Eqn 1 to describe the lifting force on warmer, less dense fluid:

$$-\eta \nabla^2 \mathbf{u} + \rho_0 (\mathbf{u} \cdot \nabla) \mathbf{u} + \nabla p = \rho_0 \mathbf{g} \beta (T - T_0) \quad (1)$$

$$\nabla \cdot \mathbf{u} = 0 \quad (2)$$

where η is the dynamic viscosity, $\mathbf{u}$ is the velocity vector, ρ_0 is the density at the reference temperature (T_0), $\mathbf{g}$ is the gravitational acceleration, β is the coefficient of volumetric thermal expansion, and T is temperature. Flow boundary conditions were a no-slip condition for the outer wall and lower boundary, a slip condition for the air-liquid interface, and an axial symmetry condition for the symmetry boundary.

Thermal effects were described by the steady-state conduction-convection heat balance:

$$\rho_0 C_p \mathbf{u} \cdot \nabla T - \nabla \cdot (k \nabla T) = 0 \quad (3)$$

where k is thermal conductivity and C_p is the specific heat capacity of the fluid. The upper and lower boundary conditions were set from experimental temperature measurements, a symmetry condition was used on the symmetry border, and an insulating condition was used for the outer wall. PDEs were solved using the finite element nonlinear solver algorithms in COMSOL Multiphysics.

Results

Radial flow velocity in a well

Steady-state flow near the bottom of a six-well plate had a radial orientation, with flow toward the outer wall. Fluorescent beads in the dish tended to sink to the bottom of the dish, where they were tracked at room temperature by time-lapse fluorescence imaging. Particle velocity from particle tracking experiments roughly 1 μm from the bottom of a well in both humid and ambient conditions (Figure 2) showed a radial dependence, with velocity increasing from the center to a maximum near 2.5 mm from the outer wall, and a roughly three-fold difference between the velocity at the center and the velocity maximum. The maximum measured outward velocity in ambient conditions was 0.72 mm/hr, and 0.60 mm/hr in humid conditions. This 20% difference suggests that evaporation plays a role in the flow mechanism.

Vertical and radial temperature gradients in a well

Two different thermal imaging techniques were used to measure temperature gradients of fluid in wells. First, confocal microscopy of a temperature sensitive fluorescent dye was used to measure the temperature in the vertical direction as a function of distance from the bottom surface.

Interestingly, the thermal gradient in the vertical direction was not statistically different ($p > .05$, two tailed t-test with equal variance) between humidified and non-humidified conditions, with an average value of $-1.3^\circ\text{C}/\text{mm}$. The temperature profiles were linear, indicating that heat transfer by diffusion dominated over convective transport, or the Peclet number (fluid velocity $\times$ length scale / thermal diffusivity) was negligible in the vertical dimension.

An infrared thermal camera was used to measure horizontal temperature gradients. The confocal microscopy technique is sensitive to micron-scale changes in distance between the focal plan and the bottom of the dish since fluid in the dish scatters the confocal laser, reducing the intensity the further the confocal laser has to travel through the liquid. It was difficult to place the dish perfectly level on the microscope stage as needed to accurately compare between different positions on the x-y plane without also slightly altering the height of the focal plane above the bottom of the dish. This would create artifacts in a horizontal scan for temperature. For this reason, infrared thermal imaging was instead chosen to measure radial temperature gradients. Thermal images were acquired at 37°C with the well placed on a metal tray in an incubator or with the well elevated such that air

contacted the bottom of the dish. The former scenario generally represents cell culture conditions in an incubator, neglecting the additional complexity of the configuration of the holes in the metal tray. The case of an elevated well with air contacting the bottom of the dish represents incubation on the stage of an inverted microscope as in time-lapse live-cell imaging, an increasingly common experimental technique. Skirted dish designs that slightly elevate the bottom of the dish close to but not in direct contact with the incubator tray were not explicitly studied in this experiment, but may be considered to fall in between these two extremes. For the case of a well placed on the metal tray, images were taken at ambient humidity (25% RH, Figure 3a), and at >90%RH (Figure 3b). Thermal images of the top (Figure 3c) and bottom (Figure 3d) surfaces of an elevated dish were acquired at ambient humidity, with the camera pointed upwards for the former and downwards in the latter case. The dish bottom was made of coverslip glass (0.17mm thick) so that the measured surface temperature would be closely aligned with the actual temperature at the bottom of the fluid layer.

Even though the average vertical temperature gradient was not a strong function of humidity under the conditions measured, it is clear that the radial gradient within the Petri dish was strongly dependent on humidity and material properties of the surroundings. Lowering humidity (Figure 3a versus 3b) caused a roughly three-fold increase in the radial thermal gradient, as did elevating the dish away from the metal tray (Figure 3c versus 3a). In the case of a dish placed on a metal tray at ambient humidity (Figure 3a), the center had the lowest temperature, but cooling was also observed at the outer wall where the evaporative flux is thought to be greatest^{13,14}. These results demonstrate that evaporative cooling leads to temperature gradients in 35-mm wells.

Meniscus geometry from confocal imaging

Data from the confocal thermal imaging experiments was also used to determine the meniscus profile. A mathematical model of meniscus shape¹⁵ was fit to experimental meniscus height data (Figure 4). Fitted parameters corresponded to a surface tension of 58 ± 2 dyne/cm and a contact angle of $17^\circ \pm 7^\circ$ (averages and standard deviations of fits from 4 experimental profile measurements). The contact angle showed significant variability between locations within a single dish. The height of the fitted meniscus profile was 1.43mm at the center of the dish and 4.33mm at the outer wall.

Finite element model of flow in a well

A finite element model of fluid dynamics and heat transfer was used to predict the flow patterns and velocity that could arise from purely buoyancy-driven flow given experimentally-derived boundary conditions. A well placed on a metal tray in an incubator was modeled with a constant bottom temperature of 37°C and an insulating outer wall. The difference between the top and bottom surface temperature at the radial center of the dish was set to 1.8°C (the experimentally-determined vertical temperature gradient multiplied by the liquid depth at the center), and the temperature of the upper boundary as a function of radius was set from the experimental temperature profile in Figure 3e. The elevated dish was modeled similarly, but with temperature at both upper and lower boundaries defined by fitted experimental temperature profiles.

Predicted thermal and flow patterns for the dish in ambient humidity at 37°C are shown in Figure 5 for the case of a dish placed on a metal tray and a dish elevated as if on a microscope stage. In the latter case, a much stronger radial temperature gradient developed, which resulted in a particle velocities (Figure 5e) two to three times that of a dish incubated on the metal tray, with the maximum outward-radial velocity increasing from 6mm/min to 12mm/min. For this simulation, temperatures at both top and bottom boundaries were set to the relative temperature fits from Figure 3c and d, assuming the same average vertical temperature gradient as in the case of a dish on a metal tray.

Symmetry in the simulation forced the flow velocity to zero at $r=0$, which was not seen in the particle tracking experiment. The non-zero experimentally observed velocity at the center of the dish may have arisen from non-radial thermal gradients that shift the location of the velocity minimum, as well as by random fluctuations, which may vary the location of the velocity minimum such that no single position has a time-averaged velocity of zero. Overall, the trend of increasing radial velocity from the center toward a maximum at around 15mm in particle tracking experiments, with a sharper peak at the maximum, was well represented by the model. However, the velocity of the particle tracking measurements is reduced by roughly a factor of 2 relative to the model. This is likely due, at least in part, to friction as the beads move along the very bottom of the dish.

The simulation results for flow in a dish incubating on a metal tray are consistent with comet length observations from influenza virus (A/WSN/33) on MDCK cells. The comet assay in Figure 1b was incubated a total of 40 hours. Virus is not released from the initial infected cells into the flowing media until around six hrs after infection (data not shown). The “comets” visualized in Figure 1b are macroscopic gaps in the cell monolayer that arise when infected cells lyse and/or detach from the plate, which begins to occur around 12 hours after they are infected (data not shown). In other words, virus spread does not occur during the first six hours, and cells infected during the last 12 hours of incubation will still be attached at the end of the experiment and will not contribute to the comets that are seen. Therefore, convective virus spread contributing to visible comets is constrained to the remaining roughly 22 hours of virus spread. At a flow velocity of roughly 0.2mm/hr to 1.0mm/hr, as seen in the simulation at 1 micron from the bottom of the dish, we would expect comet lengths between 4 and 22mm. The comets in Figure 1b have a mean length of 6.8 mm and a maximum of 13.4 mm.

Discussion

Fluid in stationary Petri dishes is subject to flow that can be relevant to the biology in the dish. For example, this study was motivated by observations that virus in liquid cultures spreads in a relatively predictable radial direction. To better understand these flows we have measured *in situ* the thermal gradients in 35-mm wells and implemented a fluid-flow model using the measured temperature profiles as boundary conditions. Two different environmental conditions of interest to biologists were investigated: 1) a dish placed on a metal tray in an incubator, and 2) a dish placed on the stage of an inverted microscope, with the bottom of the dish exposed to air, as in live cell microscopy. Cooling in the dish at the air-liquid interface, accentuated at the side wall, is consistent with evaporative cooling. A

computational flow model demonstrated that buoyancy forces arising from measured temperature gradients were sufficient to capture the radial trend in flow velocities and the location of the velocity peak at 15mm from the dish center. The particle tracking velocity at the bottom of a dish was roughly half of the simulated velocity. This low experimental velocity is likely a result of friction between the particles and the bottom of the dish.

The evaporation rate in a Petri dish depends on incubation temperature and humidity. Reducing incubator humidity from roughly 90% to 25% caused a 20% increase in particle velocity and a three-fold increase in the magnitude of the radial temperature gradients observed at the air-liquid interface. Compared with incubation on a metal tray, temperature gradients and particle velocity were increased by a factor of two to three in conditions where the dish or plate is exposed to air beneath.

There may be other factors contributing to convection in wells in addition to radial thermal gradients from evaporation in a well. For example, uneven incubator conditions¹⁶ and increased evaporation in the wells or parts of a well closest to the outer edge of a multi-well plate¹⁷ may cause non-radial heat effects. For the cases studied here the Rayleigh number (Ra) was around 200, below the threshold for thermal instability flow¹⁸, but instability flow may be relevant for different geometries. In Petri dishes, the fluid meniscus is not usually pinned, but the changing shape of a pinned meniscus has been shown to cause radial flows in the related cases of evaporating droplets¹³ and evaporating liquid in nanoliter cups¹⁹. Marangoni forces can influence flow in some liquid systems, but is likely negligible in water with contaminants or surface-active molecules at even very low levels²⁰.

Given the apparent cooling at both the center and the walls in some cases in a six-well plate (Figure 3a), the diameter of the well may be important in determining the direction of flow. For example, it is conceivable that in smaller diameter wells (e.g. 48- or 96-well plates), cooling at the wall will dominate, creating an inward flow across the floor of the well, rather than the outward flow observed in six-well plates. More study is needed to better understand flows and thermal effects in different well geometries.

The goal of this work was to characterize the flow and temperature conditions affecting biology in a Petri dish, and to better understand the origins of the flows. We have sought to measure, rather than model, temperature profiles, and use these measurements to construct a fluid dynamics model. Measurements were taken from a limited set of environmental conditions and do not represent all cases of incubating dishes. The simplifying assumptions of insulating outer walls and isothermal lower boundary conditions were sufficient for the ambient humidity case where thermal effects were strongest, but modeling of humid conditions would require a more detailed model of the thermal interactions at these boundaries. Our analysis is a step toward a better understanding of important thermal and flow conditions in this common experimental platform. This understanding could enable methods to reduce or control such flows.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We dedicate this work to youngsters Bob Bird (91) and Ed Lightfoot (90), and in memory of Warren Stewart. Thanks to Steven Loheide for advice on IR thermal imaging and use of his thermal imaging camera, Lance Rodenkirk of the UW-Madison Keck Bioimaging facility for help with confocal imaging, Kushal Sinha for technical assistance with COMSOL, Daniel Klingenberg and Michael Graham for advice on studying and modeling flows, Jay Warrick for discussions on evaporation in bioassays, and Michael Graham and Ed Lightfoot for reviewing the manuscript. We are grateful for support from the National Institutes of Health (R41-AI077296 and U19-AI0104317) and a traineeship for S.L. from the N.I.H. Biotechnology Training Program (T32-GM08349).

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Figure 1.

Virus plaque (a) and comet (b) assays stained with crystal violet. MDCK cell monolayers were inoculated with 70 PFU per well of H1N1 influenza (A/WSN/33) virus. Infected monolayers were incubated (a) 60 hours under agarose, or (b) 40 hours under liquid media, and stained for visualization. White areas represent cells killed by virus which no longer adhere to the dish. Note the characteristic circular plaque and elongated comet morphologies. For details, see (3).

Figure 2. Particle velocity in Petri dishes experimentally measured by particle tracking. One-micron fluorescent beads settled to the bottom of the dish and were tracked at room temperature by fluorescent timelapse microscopy and image analysis at five radial positions at 90% relative humidity (“humid”) and 25% relative humidity (“dry”). Flows were directed radially, toward the outer wall of the dish. Error bars represent an average of at least three 10-minute time intervals.

Figure 3.

Interfacial temperature profiles from IR thermal imaging. Thermal images were acquired in 37°C incubators of 35-mm diameter Petri dishes containing 2mL of PBS-BSA at four different conditions: (a) air-liquid interface at ambient humidity (25% relative humidity), dish placed on metal tray; (b) air-liquid interface at >90% relative humidity, dish placed on metal tray; (c) air-liquid interface at ambient humidity, underside of dish exposed to air; (d) 0.17 micron-thick glass underside of dish at ambient humidity, underside exposed to air. The temperature range between the hottest (yellow) and coldest (violet) regions is shown below the thermal images. (e) Polynomial fits of average radial thermal profiles for (a)-(d), arbitrarily set to zero at $r = 0$.

Figure 4. Meniscus curvature determined from confocal imaging. The height of the fluid layer was determined at various radial positions using confocal imaging of a dye solution (open circles). The data represents two orthogonal radial cross-sections of a 35-mm Petri dish (4 meniscus slope profiles). The physical model (solid line) is fit to the data. The vertical dashed line represents the outer wall of the dish.

Figure 5.

Finite element model of temperature and flow in a 35-mm Petri dish at 37°C and ambient humidity, with the dish placed on a metal tray (a)–(b) or elevated so that the underside is exposed to air (c)–(d). Greater temperature and flow is represented by red-brown, while lower temperature and flow are dark blue. Streamlines are shown in (b) and (d) with arrows indicating the direction of circulation. (e) Simulated flow velocity at 0.97 microns above the bottom of the dish (center plane of focal plane in particle tracking experiments) for both conditions. Experimental particle velocity data for the dry condition at room temperature from Figure 2 are shown for comparison (empty circles). Particle tracking experiments are necessarily done with the underside exposed to air. See Figure 2 for error bars. Particle tracking results give lower flow velocity than expected by simulation. This is likely due, at least in part, to friction between the beads and the bottom of the dish. Figures (a)–(d) are drawn to scale.