Lensless Stereo Microscopic Imaging

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1 Introduction

A simple inexpensive high-contrast stereo microscopic is constructed from a single video imager sensor. Two field-synchronous LEDs illuminate the subject creating disparity. The stereo microscope outputs standard field-sequential 3D video and is compatible with commercial head mounted displays and LCD shutter glasses.

The microscope has no lens, no focus, a large depth of field and no distortion between the stereo channels, reducing depth calculation to a single one-dimensional sliding correlation per object.

The easy-to-use microscope provides students and teachers with a fast and convenient way to share images of microorganisms. The microscope is compact and robust, well suited for remote sensing. The focus-free low-cost design makes it practical for a computer to continuously monitor and manage an array of microscopes for high-throughput biological experiments and research.

2 Related Work

Optical microscopes are common in biology classrooms. Students break glass slides while attempting to focus microscopes and often have difficulty understanding what they are seeing. Video microscopes allow teachers and students to share a common view but are expensive.

Several optical and lighting techniques are used to increase the contrast of images of aquatic microorganisms which are, to a large extent, transparent. Phase contrast microscopes use a set of lenses to delay the phase of light reaching the eyepiece. Dark field illumination shines off-axis light on the subject to enhance edges.

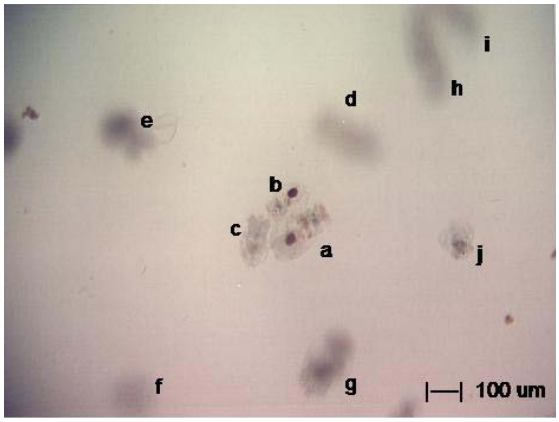

Figure 1. Rotifer in a drop of water (total thickness ~ 1 mm) photographed with a conventional optical microscope. Most of the rotifer body is transparent. The rotifer (a, b, c) in the center of the image are in focus. The other rotifer (d to i) are out of focus for they are at different depths, demonstrating the limitations of a small depth of field.

An optofluidic microscope [Heng et al. 2005] uses a mask of nanoholes above a linear imaging array to create high-resolution two-dimensional images. The subject is transported across the array using microfluidics, creating a series of image slices that are assembled into a high resolution image. The subject must not rotate as it floats by the array, restricting the output to static images.

Scientists use video microscopes to capture the two-dimensional motion of organisms in order to understanding mating, predator-pray and feeding behavior [Coulon et al. 1983, Charoy 1995, Yúfera1 et al. 2005]. Tracking aquatic organisms that move in three dimensions is difficult due to the narrow depth of field of optical microscopes (Figure 1). A single bacterium has been tracked by moving the slide holding the bacteria in three dimensions to keep the bacteria in the center of the video image [Berg and Brown 1972]. Digital In-Line Holographic Microscopy (DIHM) uses coherent laser light to track multiple organisms in 3D using a computationally-intense transform [Garcia-Sucerquia et al. 2006]. The Optical Coherence Microscope (OCM) [Hoeling et al. 2000] scans a laser beam across a subject and measures the cross-sectional scattering using an interferometer to build up a 3D image. None of these tracking techniques are simple and inexpensive.

Raskar et al. [2004] photographs inanimate objects with a sequence of flashing lights to perform depth edge detection in order to stylistically render objects. They avoid the difficulty of estimating "the full 3D coordinates of points in the scene" by performing "intensity step edge detection". In our work we must detect the 3D location of each animate

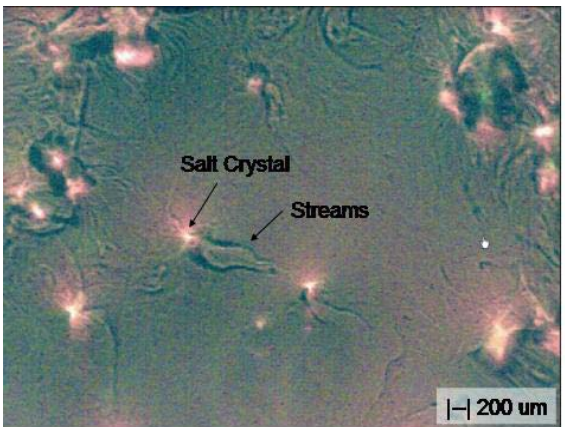

Figure 2. Salt crystals placed in a shallow pool of alcohol on top of the cover glass of a web camera's imaging sensor. Streams of salt dissolving in the alcohol are rendered visible using off-axis illumination.

object in the sensing volume.

3 Experiments with Contact Imaging

We report on the sequence of experiments that led to the development of the lensless stereo microscope.

3.1 Web Camera Experiment

The lens of a web camera is removed. An LED is placed 30 cm from the imaging sensor (imager), 30 degrees above the horizon, providing side illumination by a point-light source. A drop of alcohol is placed on the imager's protective glass cover. Salt crystals sprinkled on the alcohol create streams of salty alcohol flowing from the crystals, captured by the imager (Figure 2).

The subject (alcohol and salt) is relatively close to the imager (~ 1mm), so small changes in optical properties that alter the path of light rays reaching the imager create variations in light intensity, thereby visualizing the structure of the perturbations. The optical properties include index of refraction, density and surface curvature. This affords the lensless microscope great sensitivity resulting in high-contrast images, particularly valuable when imaging aquatic organisms like plankton that are substantially transparent.

A drop of water containing a single copepod (Tigriopus californicus, ~ 1.5 mm long) [Reed 2007] is place on the glass cover of the imager. The copepod swims around the perimeter with great speed, causing perturbation in the water captured by the imager (Figure 3). The distance between disturbances may be measured to determine stroke length in order to better understand copepod locomotion.

3.2 Megapixel Camera Experiment

The lens of an inexpensive 5 Megapixel camera is removed. A drop of water containing hundreds of rotifer (L-Brachionus plicatilis, 150 to 200 um long) [Reed 2007] is placed on the glass cover of the imager and illuminated with a surface mount blue LED placed 10 cm above the imager. The blue LED provides short wavelength illumination (470 nm) to improve contrast and image sharpness (Figure 4).

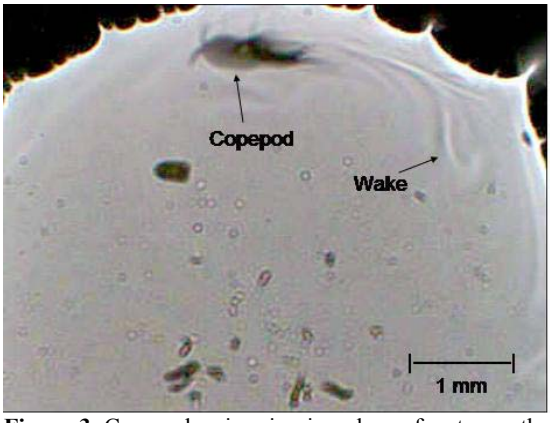

Figure 3. Copepod swimming in a drop of water on the glass cover of a web camera's imaging sensor. The wake from the propelling stroke of the copepod's feeding arms is made visible using off-axis illumination.

3.3 Video Camera Experiment

The lens of a single-board video camera [Supercircuits 2007] is removed. A 1.5 mm diameter jacketed fiber optic is placed 12 cm above the imager. The other ends of the fiber optic is inserted into a hole drilled into the epoxy case of a T1 ¾ package blue LED. A drop of water containing rotifer is placed on the imager's protective glass cover, resulting in live images of rotifer. Moving the point-light source parallel to the image plane causes the image of the object to shift. This observation leads to an innovative leap; two alternating light sources set a fixed distance apart above the imager will create pairs of disparity images that can be used for viewing and calculating the three dimensional position of objects.

3.4 Three Dimensional (3D) Experiment

A second fiber optic and blue LED is placed above the video camera, as illustrated in Figure 5. The LEDs are sequentially driven by a sync separator chip (LM1881), causing one LED to turn on during the even video field, and the other LED to turn on during the odd video field. The result is a sequence of disparity image pairs (Figure 6) output in standard field-sequential 3D format, compatible with commercial head mounted displays and inexpensive LCD shutter glasses.

3.5 Extracting 3D Trajectories

The video output from the lensless stereo microscope is recorded onto VHS video tape. The tape is digitized with VGA resolution at 60 Hz using "bob" deinterlacing to preserves the full interlace fields.

A computer program binary quantizes each field of the movie using a fixed threshold, for the image is very high contrast. Neighboring pixels are grouped together as objects. Each object in the odd field is shifted horizontally (+x

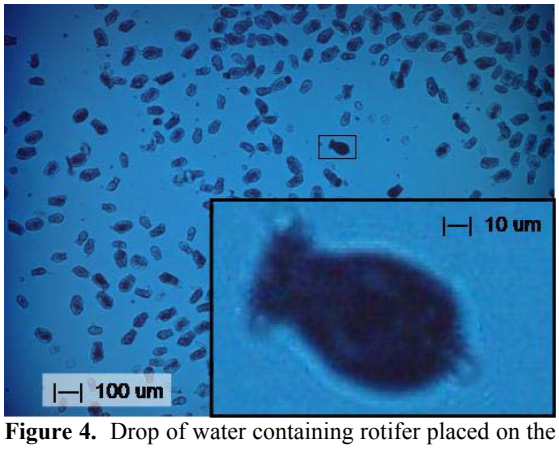

Figure 4. Drop of water containing rotifer placed on the glass cover of a 5 Megapixel imager (~2.2 um pixels) of a digital camera. A surface mounted blue LED is located 10 cm above the imager. (Insert) Zoomed view of a single rotifer.

direction) and correlated with the even field, to find the disparity (maximum correlation) between the two shadows of an object. Objects are labeled and tracked over time by measuring object overlap between adjacent odd fields. The program outputs the label and x, y, z location (in pixels) of each object 30 times per second. Figure 7 shows a 3D plot of the raw output of extracted x, y, z location (in pixels) for twenty-two rotifers swimming in a drop of water captured over 30 seconds. The light rays in Figure 5 construct similar triangles, making disparity-to-Z conversion trivial. The conversion from pixels (x, y, z) to distance (X, Y, Z) is;

$$X = x * H$$
$$Y = y * V$$
$$Z = z * H * (D/W)$$

where;

H = Pixel horizontal lenght (9.6 um)
V = Pixel vertical length (7.5 um)
D = LED to imager distance (113 mm)
W = LED to LED distance (8 mm)

4. Discussion

4.1 Calculating 3D Trajectories

The lensless stereo microscope eliminates many alignment and distortion problems encountered in 3D reconstruction of disparity images. There are no lenses and only one imager so there is no distortion or affine mismatch between the two channels. The only alignment variable is the angle between the plane of LED and the imager pixel rows which is established during microscope construction. The microscope's high-contrast images and optical simplicity makes image processing and disparity computation trivial and extremely fast.

4.2 Image Resolution

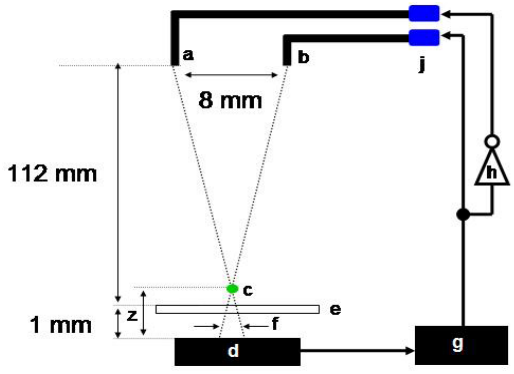

Figure 5. Light from fiber optics (a & b) sequentially illuminate object (c) casting alternating shadows on video imager sensor (d) protected by a glass cover (e). The shadow separation (f) is proportional to object's distance (z) from the imager. Video sync separator (g) in combination with inverter (h) drives LEDs (j) on alternate (even/odd) fields resulting in field sequential 3D output.

The resolution of the lensless microscope is the size of the pixels. The 5 Megapixel camera has 2.2 um pixels, providing finer resolution than the web and video imager (~8 to 10 um, respectively). Unfortunately inexpensive cameras only outputs jpeg compressed images, throwing away useful image information and resolution. The Beyer color mask built into color imagers blocks blue light from reaching three out of every four pixels, further reducing image quality. A Megapixel monochromatic imager with raw pixel output would produce superior microscopic images.

Imager venders reduce pixel size to decrease the area and cost of the imager die, limited by signal-to-noise which is proportion to pixel area. Since the lensless microscope illuminates the imager directly there is tremendous signal, implying that higher resolution imagers are possible, limited by transistor size and light diffraction by object features.

For 3D tracking, field rate and image sensing area are more significant than image resolution. Slow field rates can lead to tracking mistakes of fast moving objects. Objects can drift in and out of small sensing areas. The lensless microscope requires two fields to determine 3D location. The tracking rate is half the field rate. NTSC video has 60 fields per second, resulting in a tracking rate of 30 Hz. Other approaches that provide field rate tracking include leaving both LEDs on and matching object shadows by shape, and using colored LEDs (e.g. green and blue) to match and view object shadows by color.

5 Applications

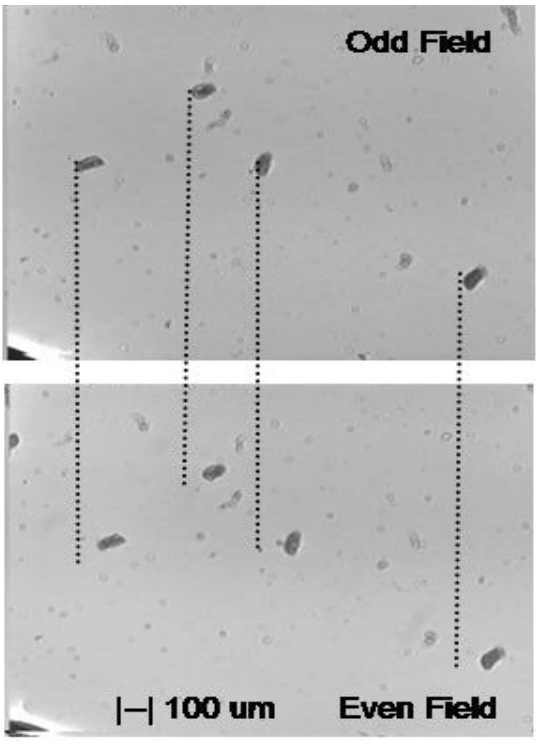

Figure 6. Pair of video fields of rotifer swimming in a drop of water ~1 mm above an imager sensor. Guide lines show image disparity caused by field-sequential illumination by a pair of LEDs mounted 113 mm above the imager.

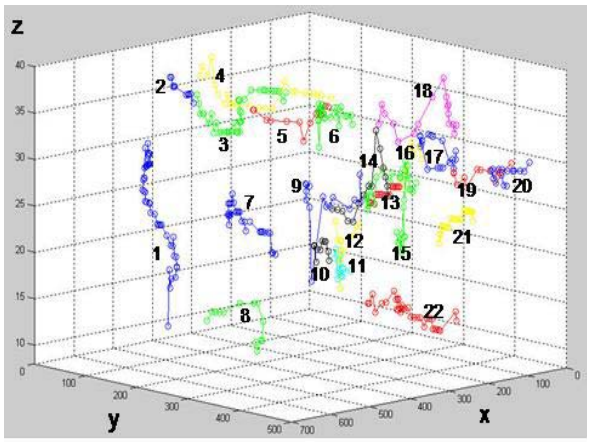

Figure 7. Three dimensional trajectory of twenty-two rotifer swimming in a drop of water ~ 1 mm above a video imager. Each circle is the location (in pixels) of a rotifer sampled at 30 Hz. Rotifer distance above the imager (z) is proportional to the disparity between shadows on the imager created by two frame-synchronous driven LEDs 113 mm above the imager.

The lensless microscope has applications in biological education and research. For students the microscope is an inexpensive and easy-to-use tool to explore the world of microorganisms. A specimen is placed on the imager and immediately viewed. When done, the cover glass is wiped clean with a lintless cloth; no glass slides to break or throw away. The microscope's sensitivity to small changes in optical properties (surface curvature, index of refraction, density) reveals structure and phenomena without focusing and staining. The light source can be moved to create dark field illumination. The output can be viewed, captured and broadcast using web camera software. A video stream can be projected and recorded. The addition of another LED and LCD shutter glasses allows students to view objects in 3D.

The lensless microscope has very few components making it compact and robust for remote sensing, on land and underwater. The inexpensive no-focus design makes it practical for a computer to continuously monitor and manage an array of microscopes for high-throughput biological experiments and research.

6 Acknowledgements

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