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VR System for Counting Biological Features
in a Volume**

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ABSTRACT

Many scientists analyze 3D biological data by counting cells and other structures when automatic algorithms cannot reliably do it. Due to the complicated nature of this data, our hypothesis is that users can count faster and more easily in a CAVE-like environment than in a Fish Tank. Our objective in this work was to determine if there was a difference between these environments in their entirety, with respect to user performance for a counting task. Both environments are standard visualization devices, and for our task we used identical wand-controlled user interfaces. To help test our hypothesis we conducted a user study in which seven subjects performed the counting task in both environments. In general, our results showed that subjects marked cells at a higher rate in the Cave and preferred working in it.

Keywords

Human-centered environment, 3D user interface, volume rendering, fish tank, virtual reality, Cave, biology, confocal laser microscopy.

INTRODUCTION

To support a collaboration with biologists, we have designed and implemented a system for studying digitized volumes of multi-channel data in close collaboration with them. Our system runs in the Cave at Brown University, which is a CAVE-like system, as well as in a Fish Tank VR environment (see Figures 1 and 2).

The quantitation of cells or cellular components is critical in the study of many biological processes. The density of cells or certain components within a particular volume is often compared between control and experimental samples. Immunohistochemical techniques that make use of antibodies tagged (or labeled) with a fluorochrome or other molecule that fluoresces when activated with particular wavelengths of light, allow biologists to specifically highlight (or recognize) the structures of interest within a tissue preparation. The digitized data is collected with a laser scanning confocal microscope (e.g., Leica TCS SP2 AOBS [15]) which generates a static volume data set.

Determining the density of cells in each data set requires two tasks: 1) identifying an equivalent sub-volume within

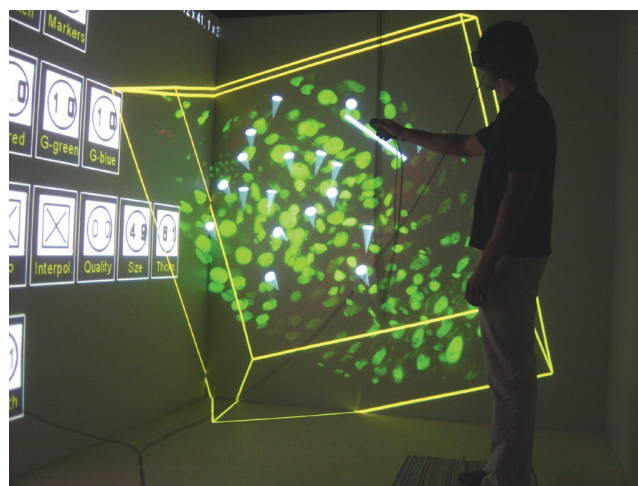


Figure 1. Viewing a cell data set in the Cave. The user places a white cone-shaped marker on a cell by reaching out to position it and pressing a button on the wand. On the left wall are several visualization controls.



Figure 2. Viewing a cell data set at the Fish Tank system. The user places a marker. We are using two monitors to provide a continuous wider field of view—the control widgets are on the right, and the data set is on the left.

each data set, and 2) accurately counting the cells in the sub-volume.

The first task requires domain knowledge to best identify and optimally register an equivalent sub-volume across samples. This might be done by using several anatomical features as landmarks for marking the boundaries in which to count cells. The second task requires uniquely identifying and tallying the cells within the volume. Here the main challenges are isolating individual cells, and due to the large number of cells, avoiding double-counting.

In this paper we describe a user study, in which users performed the second task, counting cells. This task is more easily learned by most subjects, but knowing how to isolate the correct volume to count cells in is much harder. Therefore, we provided a pre-selected sub-volume for subjects to count cells in. Nevertheless, we feel our systems that enable the user to be immersed in the 3D space would be valuable for an experienced biologist to use for identifying volumes to count cells in. In the study, we provided a user interface that lets subjects adjust the scale of the data set, change their viewpoint, and place a marker in 3D which was useful for marking which cells they had already identified. The system automatically kept count of the number of markers that had been placed. Our hypothesis is that users can count faster and more easily in the Cave than at the Fish Tank.

RELATED WORK

Most studies about the effectiveness of virtual environment at different levels of immersion have either compared extreme opposites, like a CAVETM-like environment and a standard desktop computer [1, 7, 10, 12, 14], or they have compared different screen sizes and resolutions of displays without stereo, head- or hand-tracking [5, 11, 17].

In our study, we compare two virtual environments at the high end of immersion (Cave and Fish Tank) because it is not obvious which environment will be more effective for our task. In fact, a related study by Demiralp et al. [6] found that for their task the users preferred the Fish Tank to the Cave. However, in addition to viewing and rotating a data set, our task also involved moving and selecting 3D positions.

An early volume visualization software for a CAVE, which is in many respects similar to ours, is the Crumbs system by Brady et al. [3]. It uses the same texture based volume rendering technique that we are using, but supports less functionality for volume rendering. An evaluation of the Crumbs system has been presented by Swartz et al. [16]. The Crumbs project has been discontinued, and to our knowledge the software has never been adapted to other virtual environments than CAVEs.

Bowman et al. [2] compared a CAVE-like environment to an HMD for a navigation task. In contrast to ours, their interaction did not involve 3D selection, but only navigation with a 2D joystick.

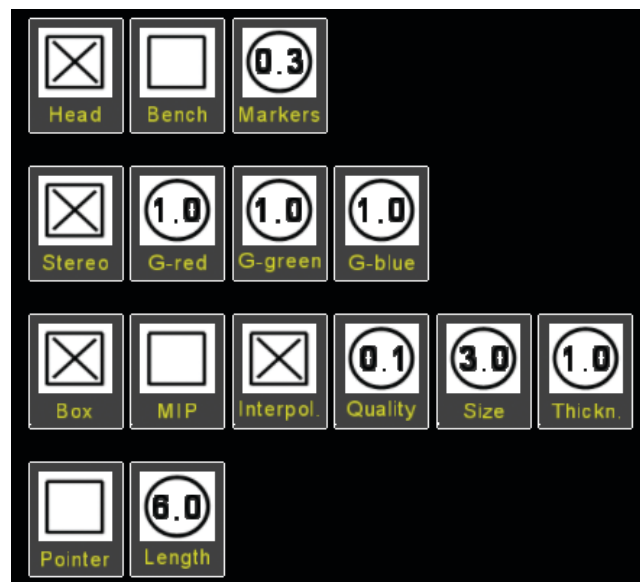


Figure 3. This user interface is shown on the left wall of the Cave and on the right monitor of the Fish Tank.

For similar reasons as Swan II et al. [8], we selected a real-world task as the basis of our formal user study. However, Swan II et al. did not work with volume data sets, nor did they use a Fish Tank environment in their study.

SYSTEM OVERVIEW

We implemented our software system in a four-sided Cave and for a dual-monitor Fish Tank virtual reality environment. Both environments use Polhemus trackers and Wanda input devices. The Wanda is a hand held input device (wand) with three buttons and a push-ball that reports relative force in two dimensions. We track the user's head and the dominant hand. Both systems provide active stereo with shutter glasses, and both are equipped with Nvidia Quadro FX 3000 graphics cards. The Cave uses the 3000G version of these cards to synchronize the four images. The resolution is 1024×768 pixels per screen in both environments. The Cave is driven by four PCs with dual Intel Xeon CPUs at 2.8 GHz and 1 GB RAM. The Fish Tank is driven by a PC with an AMD Athlon XP 1700+ CPU and 512 MB RAM.

Our software is based on an in-house library to support the rendering cluster, input devices, and volume rendering based on textured polygons [4]. We use the OpenSceneGraph API [9] for the graphical elements of the user interface.

USER INTERFACE

The central part of the UI is a set of rectangular widgets on the left wall of the Cave, or on the right screen of the Fish Tank (see Figure 3). These widgets are permanently displayed at fixed positions. We chose to display them in the plane of the screens so they are always projected at the same position, independent from where the head-tracked user is looking. Due to the unavoidable tracking lag, this gives them a much more stable feel than free-floating widgets, and even

for non-tracked users they are always in the same place and therefore easily readable. This is especially valuable in the Cave, since it is meant to be used by small groups. The widgets usually do not block the user's view because typically our users do not need all four Cave walls to view the type of biological data sets they work with.

We implemented three types of widgets to trigger actions, change a state, or to set a scalar value:

- **Action buttons** trigger an action when the user moves the pointer over them and presses the left button on the wand.
- **Check boxes** change their state when they are clicked on. An "X" or a blank space indicate the state.
- **Dials** change their value when the user clicks on them and holds the button, while rotating the hand to the left for lower values, or right for higher values. Dials are the equivalent of sliders in desktop applications, but in virtual environments dials have been found easier to use (see [13]).

FUNCTIONALITY

We explained the following components of our software to the subjects to enable them to best take advantage of the provided functionality.

Moving the data set: The data set can be moved and rotated by pointing at it and clicking the middle button on the wand. While the button is depressed, the data set sticks on the pointer like on a fork. Repeated grabbing and moving allows to place it anywhere in the virtual world. Our wand device in the Cave has a trackball which rotates the data set about its center when pushed in a direction. The Fish Tank system did not support trackball rotation. We will discuss this difference in the Discussion section below.

Reconstruction quality: Volume rendering suffers from low frame rates when a high reconstruction quality is desired, i.e., no information from the data set is skipped. In our task, the cells are sufficiently big that a lower rendering quality is possible without skipping entire cells. The subjects could change the reconstruction quality with a virtual dial on the Cave wall, which in turn changes the frame rate.

Volume size: Two dials allow setting the perceived size of the data, and separately the size along the z-axis (thickness).

Data intensity: The data set used for the tasks consisted of two color channels, red and green. To perform the task of counting cells, the green channel sufficed, the red channel did not even add any useful information. The user can change the intensity of each channel interactively, and before the tasks we set the red channel to be transparent to make sure the subjects were not distracted by it. We set the intensity of green to the same default position for all subjects, and only a few users changed this value during the tasks.

Markers: Cone-shaped markers can be placed in the data set to mark a cell so that it is not counted twice. The computer keeps track of the number of markers placed, so that the

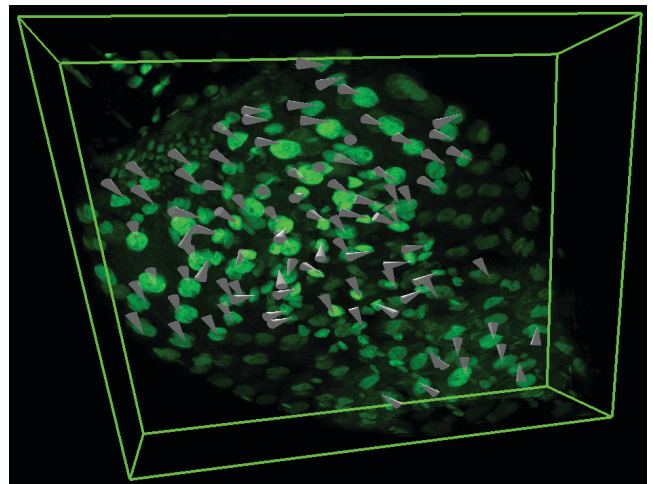


Figure 4. A subject marked 93 cells in a trial data set in the allotted ten minutes.

subjects do not have to count them. The size of the markers can be changed with a virtual dial.

Pointer length: The markers showed up at the end of a virtual stick, which seemed to come out of the tip of the wand. The length of the stick can be changed with a dial. Shorter lengths allow more intuitive and direct marker placement, especially at low frame rates. A longer pointer, however, allows the user to move his hand around less and move the marker by merely turning the hand, instead of moving it. The latter proved to be particularly useful in the Fish Tank environment, where the range of motion is naturally limited by the sitting posture and the smaller space between the user and the screen, as opposed to the Cave.

EVALUATION

Seven students (4 male, 3 female) participated in our formal user study: we recruited five undergraduate and graduate students from computer science, one undergraduate political science student, and one Ph.D. candidate from the biology department. All but one subject had no or very little experience with CAVEs or Fish Tank systems. The Ph.D. candidate has often used the system in the Cave before (except the marking function), but had not previously seen the Fish Tank. However, this user did not finish the experiment in the Cave, so we did not include her data in the statistical analysis.

The user study took place on four days. Each of the participants spent a total of about 1.5 hours in our lab and was paid \$15 for their participation.

The subjects first filled out a consent form and a short questionnaire, asking for statistical information like age, gender, and previous experience with virtual environments. Then we explained them the task: they were about to see two data sets from a confocal laser microscope depicting cells of the larva of fruit fly (*Drosophila*). The subjects should count the cells by marking their visible nuclei with cone-shaped

markers. The computer kept track of the number of markers placed. The markers help prevent counting cells multiple times, and to know which ones had already been counted. Figure 4 shows one user's data set when he was finished with the trial in the Cave.

The task was divided into four sections: one practice and one trial run in each of two virtual environments, the Cave and the Fish Tank. Two different data sets were used throughout the study: in each practice run the participants used the practice data set (see Figure 5a), and in each trial run they used the trial data set (see Figure 5b).

Each subject first had a practice and a trial run in one virtual environment, and then a practice and a trial run in the other. We switched the order of environments for each subject to prevent ordering effects: if one subject started in the Cave, then the next subject started at the Fish Tank.

The trial runs were timed to 10 minutes. The practice runs were not as strictly timed, but we suggested to spend about 10 minutes and after this time we asked whether the subjects felt comfortable using the system. If they did not, we allowed them additional time, but most subjects did not need it.

The subjects were encouraged to ask questions about how to use the system at any point, because we did not want to test how well they could memorize the system's functions. If we noticed that subjects struggled with something because they forgot about a system feature, we helped them no matter which run they were in. In the trial runs we never entered the Cave but only gave spoken comments.

After the final run, the subjects were given a two-page questionnaire with ten questions to rank Cave and Fish Tank, along with five free questions. In the ranking questions we asked them to rank the two environments according to several criteria. For each question, there were seven boxes to fill in the first letter of the environments ("C" for Cave, or "F" for Fish Tank), thus ranking each environment on a scale from 1 to 7.

In the free questions, we asked the subjects for their thoughts on a variety of other issues. The study ended with a short debriefing, in which we summarized the goals of the study.

In the following section, we will list the questions we asked, and we will summarize the answers we got. We will also present an analysis of task performance, and our observations while the subjects worked in the virtual environments.

Results

Our user study produced three types of results. The first is task performance, which we analyzed statistically. The second type are results we obtained from the questionnaires. The third type are observations we made during the tests. We will present all these results in the following sections.

Task Performance

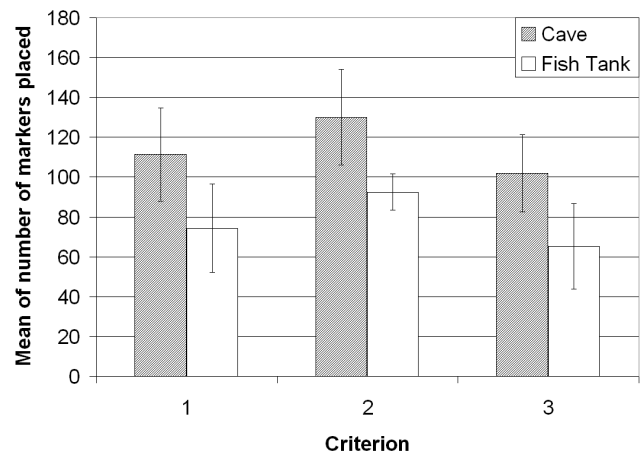


Figure 6. The left bars show the mean of the number of markers placed in the Cave, the right bars show the mean for the Fish Tank. The three criteria are: 1=over all subjects, 2=only subjects who were in the Cave first, 3=only subjects who were at the Fish Tank first. The range indicators are the standard deviations.

From the seven participating subjects, we use only the results of six of them for a statistical analysis, because one user did not finish the experiment. Our dependent variable is the number of markers they placed in each of the two environments. We did a t-test for within-subjects experiments, and a within-subjects test for repeated measures to check for ordering effects. In both cases we checked for significance at the 5% level. The null hypothesis is that there is no difference in how many cells subjects mark in the two environments.

In general, we found that our subjects marked more cells in the Cave. Our t-test analysis shows that our result is statistically significant with a p-value of 0.012. The t-value is 3.811. Criterion 1 of Figure 6 shows the mean values of all subjects in Cave and Fish Tank, along with the standard error bars.

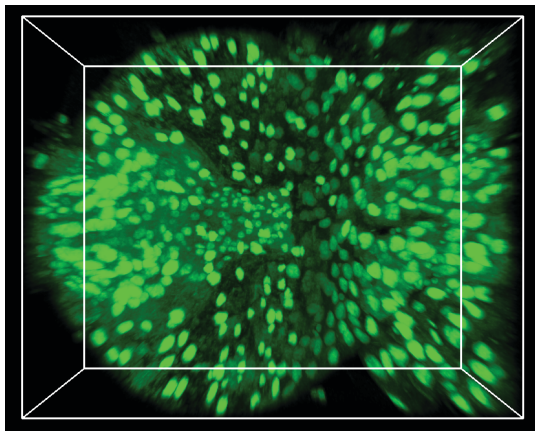
A test for repeated measures shows that there are no significant ordering effects. Criteria 2 and 3 of Figure 6 show mean and standard deviation of the measurements grouped according to which environment was first: Criterion 2 considers only those cases in which the subjects started in the Cave, Criterion 3 considers those cases in which they started at the Fish Tank.

Figure 7 shows the number of markers for each subject.

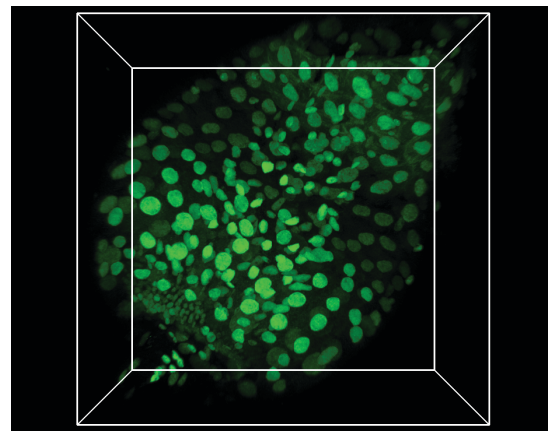
Questionnaire

The subjects filled out two questionnaires: one before the task performances ("pre-questionnaire"), and one after ("post-questionnaire").

In the pre-questionnaire, we asked the subjects about their age (average: 20.5 years, standard deviation: 1.0), sex (4 male, 2 female), and their dominant hand (all right handed). We asked for their concentration or job title: one was a politi-



(a)



(b)

Figure 5. The two data sets we used in the user study. Both are scans of confocal laser microscopes, showing parts of internal organs of *Drosophila* larvae. (a) is the data set we used in the training, (b) is the one we used in the trial.

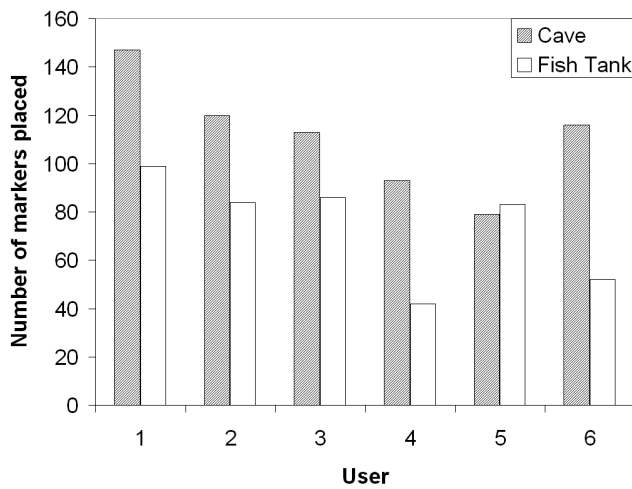


Figure 7. The pairs of bars show the number of markers the subjects placed in each environment. The left bars are for the Cave, the right bars are for the Fish Tank.

cal science undergraduate student, the others were computer science students (4 undergraduate, 1 graduate student). We also asked for vision deficits: all but one of the subjects answered they had deficits, but they were wearing contact lenses or glasses.

We also asked for prior experience with 3D graphics programs: two subjects had minimal to no experience, four had previously used such programs in class or at home. To our question for prior experience with virtual reality systems, two subjects answered they had used virtual environments once, one had used a power wall in the past, the remaining ones did not have previous such experience.

In the post-questionnaire, we asked the subjects to rank Cave and Fish Tank according to ten criteria, and we asked five free questions. Table 1 lists all the ranking criteria and shows average values and standard deviations of the results.

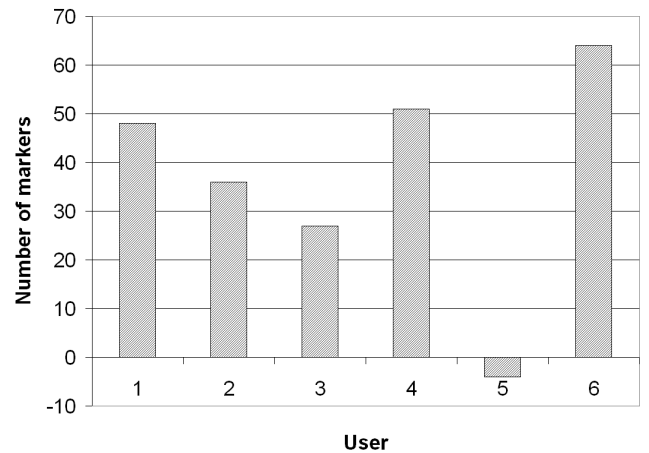


Figure 8. For each user the bars show the markers placed in the Cave and at the Fish Tank (Cave minus Fish Tank).

The Cave scored consistently higher than the Fish Tank. Only rarely did subjects rank the Fish Tank higher than the Cave: two subjects at criterion 5, and one each at criteria 4 and 10.

After the ranking questions, we asked five free questions. In the following paragraphs we will present the most interesting answers that we got.

Question 1: “What do you think should be done to improve the effectiveness of any of the systems?”

One user suggested “some kind of scroller” to adjust the length of the pointer, because he changed it often. He also suggested that we “take into account the gap between the screens” at the Fish Tank: we were using a virtual desktop that extended over both monitors, which resulted in a slightly offset picture on the right monitor. Two subjects suggested, independently from one another, to include the non-dominant hand in the interaction. One suggested to “put the navigation in it”, another suggested to have it “control rota-

Table 1. Post-questionnaire ranking criteria and results: average and standard deviation (in parentheses)

#	Criterion	Cave	Fish Tank
1	Ease of learning how to use the system	5.8 (1.2)	3.7 (1.0)
2	Help provided by the system to perform the task	5.2 (1.5)	3.7 (1.0)
3	Ease of use once you had learned how to use the systems	6.3 (0.8)	4.0 (1.4)
4	The speed you experienced to perform the tasks	6.2 (1.2)	3.8 (1.5)
5	The perceived quality of the displayed data	5.2 (1.2)	4.3 (1.5)
6	The effectiveness of the user interface	5.7 (1.0)	3.3 (1.0)
7	How comfortable were you using the systems	6.7 (0.5)	3.0 (2.4)
8	How confident were you when using the systems	7.0 (0.0)	3.8 (2.4)
9	How much did you enjoy working with the systems	6.7 (0.5)	3.8 (1.6)
10	Your preference if you were a professional	6.2 (1.3)	3.0 (1.7)

tion, length, width of data, etc, as you would change modes in Photoshop”.

Question 2: “Is there anything you especially liked or disliked with any of the systems?”

One user liked about the Cave “the opportunity to put my hand through the image”, and another one liked the “ability to immerse yourself in the object”. Two subjects disliked about the Fish Tank that the “angle of my hand was awkward” or “in less natural positions than in the Cave”. One user “disliked marking data points that were behind others” in the Fish Tank. Another one disliked about the Fish Tank “not being able to see the entire pointer—all you could see was on the actual screen”.

Question 3: “Do you think we are doing a fair comparison of the systems? If no, please explain why not.”

All but two subjects thought the comparison was fair. One of the others reported: “I think if the Fish Tank had better sensors it would score slightly higher.” The other reported that not having the trackball at the Fish Tank made it an unfair comparison.

Question 4: “Did you feel disoriented or discomfort at any time during the experiment? If so, please briefly describe.”

Two subjects reported that their arms and wrists got tired in the Fish Tank. One user got dizzy enough in the Cave experiment that she had to abort the experiment after about 7 minutes; we did not use her data for any of our analysis. One user “felt frustrated and annoyed at my inability to maneuver as I wanted” at the Fish Tank. One user reported that at the Fish Tank he was “positioned uncomfortably”.

Question 5: “If you have any additional comments concerning your experience in this study, please feel free to provide them here.”

To this question we mainly got positive comments on how much the subjects liked the Cave. One subject suggested to add a “long distance shot” of the data set to the existing visualization, because “it is hard to get a good view of the screen when you are up close”.

Observations

We were surprised at how quickly all candidates learned how to use the system, no matter if they started at the Fish Tank or in the Cave. The about ten minutes practice time turned out to be enough in all cases, in fact in the second environment, some of the participants told us they were ready to start with the trial before the practice time was up.

There were considerable differences in how the subjects worked with the data sets in both environments. One of them, who did not have previous experience in virtual environments, moved around much more than the others. At the Fish Tank, he moved the entire arm with the wand, and he even got up from his chair at some point to see if he could work better when standing (he figured he could not). Note that this was even before he was in the Cave. During the practice run in the Cave he walked around most of all participants, and moved the data set to many different places in the Cave. When asked why he did this, he said he wanted to see what it looks like from a different perspective.

In the Cave, after having set up all viewing parameters, most of the other subjects ended up standing in the middle of the Cave while performing the trial task, not moving their bodies much and focusing on marking the cells. Differences were in the size at which they viewed the data set, the size they set the markers to, and the length of the pointer, i.e., the distance of the hand from the newly to be placed markers.

At the Fish Tank, the subjects were naturally much more stationary than in the Cave, as they were sitting in front of the two monitors. They could rest their arms on the chair’s armrests, but many of them did not take advantage of this for most of the time because that posture did not allow them to reach everywhere they wanted. Interestingly, all subjects preferred to place the data set behind the plane of the screens, even after having been suggested to move it closer to reach it better. Therefore, they had to set the distance from wand to marker to a higher value than otherwise, which required more effort to precisely place the markers.

It was interesting to see that most subjects selected a higher rendering quality at the Fish Tank than in the Cave. Even though this resulted in lower frame rates at the Fish Tank, they preferred their settings, despite us making them aware of their options. In the Cave, the subjects set the quality level

so that they got about 8 frames per second (fps), at the Fish Tank they were happy with about 4 fps.

The subjects consistently chose longer pointers at the Fish Tank than in the Cave. In the Cave, most subjects preferred the marker to be as close to the hand as possible, without blocking it with the hand. At the Fish Tank, all subjects placed the data set behind the screen, so that the distance between hand and marker was larger than in the Cave.

DISCUSSION

Rendering quality. We were surprised at most subjects giving rendering quality higher preference at the Fish Tank than in the Cave. We hypothesize that due to the Fish Tank's crisper display (more pixels per inch and clearer pixel boundaries), the artifacts are more noticeable at the Fish Tank than in the Cave and thus the human vision system cannot as easily ignore them and create a continuous image. This might be similar to a TV image which looks fine on a large but low resolution TV screen, but looks pixelated on a smaller but higher resolution notebook screen. In the Cave, the CRT projector technology slightly blurs the image.

Confidence. Another interesting result is that the subjects consistently scored the Cave higher in confidence than the Fish Tank. We hypothesize that this is due to the more natural interface in which they could use their whole bodies to move around in the virtual world and felt higher presence. Many subjects made the data sets big enough in the Cave that they could put their hands inside the boundaries of the data set. At the Fish Tank, all subjects chose to put the data set behind the screen, which did not allow them to put their hands in the data set. This, too, might have added to higher perceived confidence in the Cave. Another reason could have been the lower frame rate the subjects chose at the Fish Tank: the therefore higher latency, along with the longer distance between hand and marker that they chose, allowed a less direct placement of markers and required more visual feedback to accurately place markers.

Trackball. As stated earlier, the trackball on the wand which rotates the data set around its center, worked only in the Cave. However, because we observed that subjects spent little used the trackball in the Cave, which happened before we timed the trial, we think one can neglect the impact of this on the study. The data sets had ten times more detail within the slices than along the z-axis, so rotation was not necessary to achieve good task performance.

Cave preference. It was surprising to us that the Cave scored considerably higher in most rankings than the Fish Tank. We had not anticipated this after the study by Demiralp et al. [6]. What might be the reasons for this? We believe that it is due to the fundamental differences in the tasks. In [6], the data set is solid and only has features to look for on its surface. By rotating it the subject can see all he needs to move on, and he does not need to interact in any other way than rotating it. So only three degrees of freedom of the input device are actually used, whereas our task required control over all six of them. Our data set has features (cells) all over

the place, many of them can only be seen at specific viewing angles because they are blocked by other cells. This requires much more careful control over the viewpoint. Additionally, placing markers near these small features requires very fine movements of the wand, which were not required in [6]. On the other hand, it is possible that the subjects were overwhelmed by the wow effect of the Cave, which most of them had never seen before. We tried to make the subjects aware of the goals of our study to get results independent from this possible influence, but we will need to run more studies with differently experienced people to factor it out entirely.

Arm fatigue. We did not expect the subjects to complain more about arm fatigue when using the Fish Tank than in the Cave. However, one subject said that he "could work in the cave all day" but at the Fish Tank only for about 15 minutes because of arm fatigue. We hypothesize that our method of pointing and clicking at features worked better in the Cave than at the Fish Tank because the hand was part of the virtual world, at the exact location where it was in the real world. In order to see where the hand is, the subject would have to hold it up in front of him because this is where the screens are, but that is obviously an uncomfortable position in the long run. In the future, we will experiment with actual hand positions that are offset from the virtual hands so that the users can leave them in a more comfortable position. We expect that this will require a similar learning effort as what people experience who use a desktop mouse for the first time: it requires some getting used to before one can intuitively move the pointer on the screen while the mouse is located offset from it.

Subject marking strategy. In the Cave, subjects scaled the data set up so individual cells were about 4-6 inches in diameter. However, at the Fish Tank they scaled the data such that cells were under one inch in diameter. We think that with our interface bigger features are easier to mark, but that you only want to make them big if you have a large field of view and a large interaction space. Both conditions are features of the Cave, but not of the Fish Tank.

CONCLUSION AND FUTURE WORK

Our objective in this work was to determine if there was a difference between the Cave and Fish Tank environments in their entirety, with respect to user performance for a counting task. Our results showed that subjects could mark cells more quickly in the Cave than at the Fish Tank, and most subjects preferred the Cave.

In the future, we want to determine the specific causes of the difference by carefully controlling the experimental variables, such as screen size and field of view.

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