Nate Evans
BIOE499
Eric Seibel & Ronnie Das
3/18/15

Development of an Optical Projection Tomography Microscope Cartridge for High-resolution Imaging of Large Diameter Tissue Specimen

---

Background

Needle biopsies are commonly used in healthcare to diagnose a wide variety of medical problems and are an effective method of identifying diseased tissue. The current method of needle biopsy diagnosis requires time intensive preparation and results in two dimensional slices of the acquired tissue, which leaves three dimensional features between slices to be inferred by the health care professional.

Optical Projection Tomography (OPT) Microscopy (OPTM) is an imaging method that can, with the aid of computational reconstruction, generate extremely high resolution three-dimensional (3D) representations. Past research has shown the capacity to reconstruct 3D cellular features, using extended Depth of Field (DOF) images collected from 180 degrees with a resolution of 0.9 μm [1]. This resolution and resulting 3D cell structure shows promise for healthcare and research applications.

One previously developed device that uses the OPT technology is the CCT05, designed and built by Visiongate, Inc. This device utilizes OPTM to image cells suspended in a high viscosity, optically matched fluid. The CCT05 uses a small cartridge to suspend and rotate cells during imaging. The cartridge is comprised of an outer casing, which holds a glass capillary in the viewing plane with a high degree of precision. The precise motion control is made possible by laser etched grooves on a glass plate in which the capillary rests. The capillary has an inner diameter of approximately 50μm, which is an effective size for cell imaging.

Question/Problem

A number of technical hurdles have dissuaded past attempts at high-resolution OPTM imaging of large diameter objects. Large diameter objects in this context refers to sizes greater than 100 microns and less than 1 mm. OPTM requires high magnification objective lenses with inherently high numerical apertures (NA). The high NA objectives result in an exceedingly shallow DOF for each image and thus require computational focus stacking

methods to extend the DOF. To do this, the cell is imaged at a number of focal positions, effectively scanning through the cell. These images are then combined into an extended DOF image. The algorithms for creating extended DOF images and the filtered backprojection reconstruction, used to create 3D images from a set of 2D images, are computationally intensive. To apply OPTM techniques to image large objects therefore requires significant computing resources. Another limiting factor arises because the high NA of the objective lenses leads to the focal range being intrinsically short. When acquiring focus stack images of an object larger than the objective lens focal range, the objective lens nose will collide with the capillary before the focal plane reaches the specimens far side. These limitations have previously inhibited similar resolution OPTM imaging of multicellular tissue.

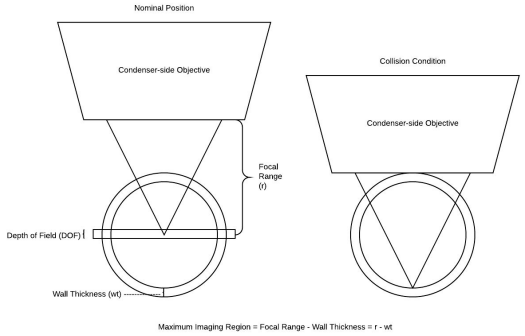

Figure 1: Nominal and Collision conditions showing how an OPT microscope's objective NA limits the size of the object it can image.

Dr. Das and the Human Photonics Lab has suggested a method using OPTM to create 3D representations of large diameter multicellular tissue, such as needle biopsies. To do this, extended Depth of Field images can be created by methods similar to focus stacking, thereby eliminating the narrow DOF inherent to OPTM. Furthermore, by means of image stitching algorithms, the reconstructed image can extend axially. To meet the computational requirements in real time, we propose to use High Performance Computing (HPC) resources such as the University of Washington's supercomputing system HYAK. These suggestions outline a path to image much larger specimens while maintaining extremely high resolution. The resulting proposed technique is optimal for long cylindrical geometries. This technique could be used for rapid imaging and subsequent analysis of needle biopsies by trained healthcare professionals. The resulting isotropic 3D representation may be a more effective visual representation for healthcare professionals to use in their diagnostic procedures than current 2D visualization techniques. Additionally, Dr. Das has proposed the use of optically clearing agents during tissue preparation. By optically clearing tissue, researchers can match the tissue's refractive index (RI) to the surrounding solution, thus making key features more observable.

To show proof of concept for high resolution, large specimen imaging, Dr. Das required an OPT microscope compatible with large tissue geometries. His previously used OPTM device, the CCT05, uses a capillary with an inner diameter of 50 μm. To remedy this limitation, we set out to develop a new cartridge, compatible with the CCT05, that implements a large diameter capillary tubing capable of accomodating multicellular tissue. The following sections detail the design and manufacturing of a novel CCT05 cartridge for use in proof of concept OPT imaging of high resolution large diameter tissue samples. This report does not document the imaging experiment and, at the time of writing this, OPT imaging using this adapted cartridge had not yet been attempted.

Method

The design of this cartridge consists of three distinct portions: Cartridge Casing, Capillary Guide and Capillary assembly. During experimentation, these three portions will also require tissue sample, a mechanically fixing medium such as agarose gel and optical coupling oil. The Cartridge Casing and Capillary Guide assemblies were modeled using computer aided design (CAD) and manufactured by either CNC mill or micro-lathe. Design of a CCT05 cartridge for Dr. Das's proposed experiments required implementation of several key features. The foremost feature was a capillary with an inner diameter capable of containing a multicellular tissue sample. To do this required a redesign of the capillary assembly and capillary guide assembly to match the enlarged capillary geometries and maintain stable rotation during imaging. Secondly, Dr. Das wanted to test a variety of optical clearing agents and mechanical fixing mediums. The RI of the optical clearing agent, mechanical fixing medium, optical glass and optical coupling fluid must be within 0.02 [1] of each other to minimize optical distortions. This required that there be distinct configuration for each combination proposed. To meet this requirement, the cartridge was made modular and easily cleaned. The major feature that makes this possible is the redesign of the capillary assembly to add a compartment wherein the capillary passes through. This allows the user to use any fluid for optical coupling and interchange capillary assemblies with various glass types and sample preparations.

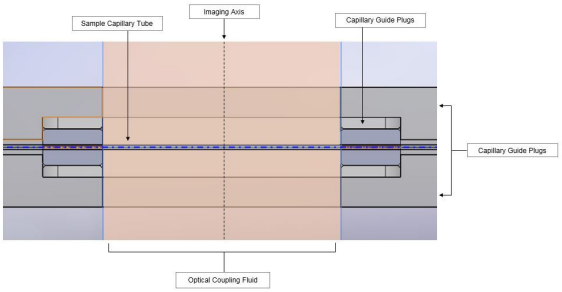

Figure 2: Detailed cross-sectional view of CCT05 specimen cartridge redesign. Corresponds to same plane as as B-B in FIG. 3.

The Cartridge Casing was further separated into multiple components to create a modular design to encourage part replacement, rapid augmentation and design iteration. The mating surfaces, on which the capillary guide fits, required close parallel tolerances to assure accurate capillary positioning and these portions were purposefully machined for an interference fit of 0.001" to increase stability of the assembly during use. Additionally, these mating features for the capillary guide were done in a single setup on a CNC mill to achieve tight dimensional tolerances. The Cartridge Casing profile was designed to imitate the original CCT05 cartridge in order to maintain the required mating features necessary for compatibility with Visiongate's CCT05 OPT microscope.

The Capillary Guide assembly is responsible for maintaining stable and repeatable rotation during image acquisition and thus required tight manufacturing tolerances. By making this assembly modular, the cartridge casing can be used with a variety of interchangeable

capillary guide configurations. The Capillary Guide consists of a rectangular section of quarter inch polycarbonate sheet and two delrin press-fit plugs. An inner section was machined out to create a compartment for optically matched fluid. On a micro-lathe, two identical plugs were machined to match the capillary outer diameter. The purpose of these plugs are to constrain the capillary tube during rotation and image acquisition. Non-circular rotation, elliptical or otherwise, results in poor image quality after reconstruction. Therefore, it is critical that the capillary axis has minimal positional deviation during rotation. To maintain capillary stability, the guide holes were drilled using either a #74 or #80, depending on the configuration. These plugs were then press fit into the Capillary Guide. Initial attempts to drill guide holes in the polycarbonate, rather than having press-fit plugs, resulted in warping of the plastic, and non-linear holes. To remedy this, the plug was manufactured from a material that could hold tighter tolerances. Both delrin and aluminum plugs worked equally well although Delrin was ultimately chosen. During operation, to further increase the concentricity of the capillary in the guide holes, an oil should be applied to the plugs inner diameter; Oil at the capillary-plug interface takes advantage of the self-centering characteristic caused by the oil's surface tension and will function to dampen environmental vibrations. This oil type and viscosity will require additional testing.

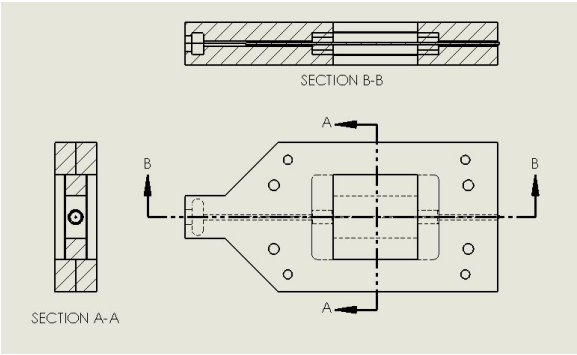

Figure 3: Cross-sectional views, A-A corresponds to FIG. 4 B-B corresponds to FIG. 2.

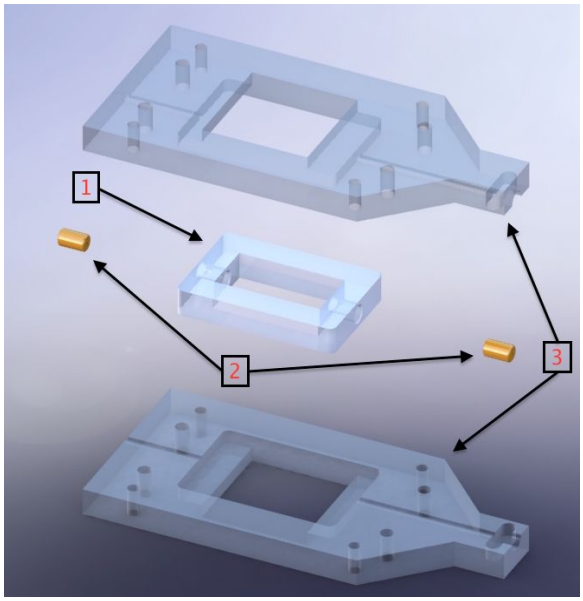

Figure 4: SolidWorks rendered model exploded view of the capillary cartridge. (1) capillary guide (2) capillary plug(s) (3) cartridge casing(s). Capillary Assembly not included, see FIG. 6.

The capillary assembly remained identical to the original design except that the capillary itself was upgraded to a larger inner and outer diameter. Two capillary assembly configurations were manufactured to give Dr. Das the option of a 0.4 mm ID or a 0.2 mm ID. These Capillary assemblies required distinct Capillary Guide configurations to fit the different capillary OD. The Capillary assemblies had an OD of either 0.55 mm or 0.33 mm.

Sample preparation requires suspending the tissue in an agarose gel or a high viscosity fluid to mechanically fix it in place within the capillary ID. It is proposed that an optical clearing agent be added before or during agarose suspension to make key tissue features more observable after imaging. This gel and optical clearing agent must be optically matched to the surrounding capillary glass to

minimize optical distortions. Additionally, an air-glass interface at the capillary will result in lensing characteristics and negatively impact imaging. To negate this, the capillary is usually constrained in a square glass capillary. In lieu of this technique, we have proposed to fill the region surrounding the capillary with an optically matched fluid so that the light path is orthogonal to the air-fluid interface. The optical clearing agent, glass type and optical coupling fluids must all have similar refractive indices to be optically coupled well. This limits the usable capillary glass type and optical clearing agent combinations. After careful search, two functional combinations are proposed:

1) Duran glass tubing (n = 1.473), made by Schott in combination with Glycerol (n = 1.4716)
2) SCHOTT8252 (n = 1.538) with methyl Salicylate (n = 1.538).

Optical oils are widely available with refractive index resolution of n = XXX +/.001. Glass coverslips are glued to the capillary guide surfaces to contain the optical coupling fluid. The air-coverslip and coverslip-fluid interface surfaces are both orthogonal to the light path and therefore the coverslip glass type can be arbitrarily chosen, however, high quality glass should be used to minimize light scattering.

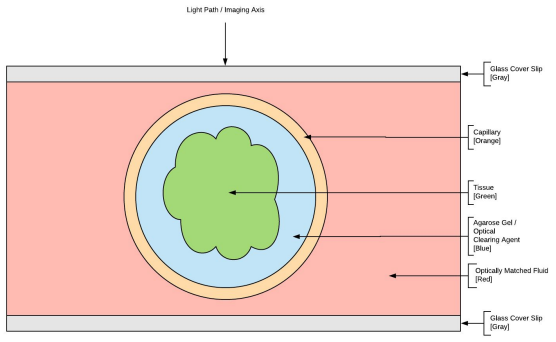

Figure 5: An artist's representation of the tissue cross-section view showing the light path and optically coupled components. Shares the same plane as A-A in FIG. 3.

The materials used for this project were carefully chosen. Clear polycarbonate plastic was used so that the inner workings of the device would be visible to the user, making loading and unloading easier. Additionally, polycarbonate was chosen for it's ease of manufacturing and impact resistance. Fused Quartz Glass Capillaries were ordered from VitroCom, Inc. Their diameters were chosen based on the limitations of the CCT05 OPT microscope focal length; If the capillary OD is any larger than 0.55 mm, the focal plane of the OPTM could not reach the far side of the capillary ID without collision between the objective lens's nose and capillary OD. Because of this limitation, we chose a capillary ID of 0.55 mm, thus maximizing the usable tissue diameter at 0.40 mm. A second configuration was manufactured with an ID of 0.20 mm and a OD of 0.33 mm.

Observations

There were several observations worth noting. First, the polycarbonate had a tendency to scratch easily and, as seen in Figure 7, scratches were acquired during the manufacturing process. This could be remedied by more diligent storage of the materials, careful machining practices, or by using a different material. Furthermore the tolerances have not yet been assessed and more precise machining may be necessary to meet the needs of this project. The modular nature of this device makes further augmentation easily applicable, including rapid capillary diameter change and view field augmentations.

Results and Discussion

Independent of machining tolerances, the capillary plug holes, drilled with #74 and #80 drill bits, corresponding to 0.5715 mm and 0.3429 mm respectively, were the closest widely available bit size to match our desired capillary ODs. This leaves a capillary guide gap of 21.5 microns in the 0.55 OD configuration and 12.9 microns in the 0.33 OD configuration. These configurations guide gap allow a maximum capillary axis deviation of more than 10 microns, which would lead to a poor reconstruction. Axial movement of the capillary during rotation must be exceedingly small for the OPTM to achieve high quality 3D reconstruction and until this device is tested, we are unsure if this meets the project's requirements. One possible solution to axial wandering is to immerse the capillary tube in oil at the interface between the plug and capillary. The self centering nature of the oil has the potential to increase capillary stability during rotation. Additionally, it may also be valuable to use a ferromagnetic liquid at this interface in combination with a variable magnetic field. By doing this, we may be able to effectively immobilize the capillary in between angular rotations so as to minimize capillary movement. Past research has shown that applying static or alternating magnetic fields to ferrofluids can change the fluid viscosity, or even induce negative viscosities [2]. By increasing or decreasing the viscosity at our plug-capillary interface, we may be able to influence the capillary stability in a positive way.

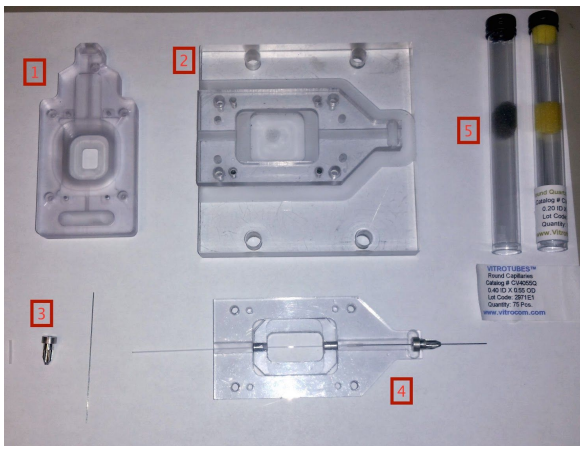

Figure 6: (1) Original cartridge design. (2) Machining plate with ½ cartridge casing. (3) disassembled capillary assembly. (4) Exposed capillary guide with capillary assembly and fit in capillary casing (5) Capillaries.

To image larger objects than proposed in this paper may be possible but would require drastic redesign of the underlying OPT microscope, deviating significantly from the CCT05. The magnification of the CCT05 yields extremely high resolution images but the optical components that make this possible, specifically the objective lenses, are only commercially available with high NA and thus a short focal range. Therefore, the only way to image larger specimens by OPTM is to decrease the magnification, thereby sacrificing resolution. If a high magnification objective lens is developed with a lower NA, then this OPTM can be applied to image correspondingly large objects.

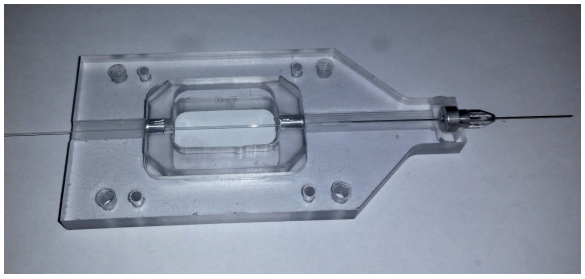

Figure 7: Exposed capillary cartridge with a 0.55 OD capillary assembly loaded.

For CAD and/or additional manufacturing details, please contact nathaniel.evansj@gmail.com

References and links

[1] Mark Fauver, Eric J. Seibel, J. Richard Rahn, Michael G. Meyer, Florence W. Patten, Thomas Neumann, and Alan C. Nelson, "Three-dimensional imaging of single isolated cell nuclei using optical projection tomography," Opt. Express 13, 4210-4223 (2005)

[2] Murray, Michael F. “Emergent Viscous Phenomena in Ferro uids.” (2008).