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Beyond isolated cells: microfluidic transport of large tissue for pancreatic cancer diagnosis

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Abstract

For cancer diagnoses, core biopsies (CBs) obtained from patients using coring needles (CNs) are traditionally visualized and assessed on microscope slides by pathologists after samples are processed and sectioned. A fundamental gain in optical information (i.e., diagnosis/staging) may be achieved when whole, unsectioned CBs ($L = 5\text{--}20$, $D = 0.5\text{--}2.0$ mm) are analyzed in 3D. This approach preserves CBs for traditional pathology and maximizes the diagnostic potential of patient samples. To bridge CNs/CBs with imaging, our group developed a microfluidic device that performs biospecimen preparation on unsectioned CBs for pathology. The ultimate goal is an automated and rapid point-of-care system that aids pathologists by processing tissue for advanced 3D imaging platforms. An inherent, but essential device feature is the microfluidic transport of CBs, which has not been previously investigated. Early experiments demonstrated proof-of-concept: pancreas CBs ($D = 0.3\text{--}2.0$ mm) of set lengths were transported in straight/curved microchannels, but dimensional tolerance and flow rates were variable, and preservation of CB integrity was uncontrolled. A second study used metal cylinder substitutes ($L = 10$, $D = 1$ mm) in microchannels to understand the transport mechanism. However, CBs are imperfectly shaped, rough, porous and viscoelastic. In this study, fresh/formalin-fixed porcine and human pancreas CBs were deposited into our device through a custom interface using clinical CNs. CB integrity (i.e., sample viability) may be assessed at every stage using an optomechanical metric: physical breaks were determined when specimen intensity profile data deviated beyond $x_{\text{avg}} + 2\sigma$. Flow rates for human CBs were determined for several CNs, and microfluidic transport of fresh and formalin-fixed CBs was analyzed.

Keywords

3D pathology; tissue microfluidics; core biopsy transport; optical imaging; sample preparation

1. INTRODUCTION

1.1. Cancer, diagnosis and pathology

Cancer currently is the second-leading cause of death for Americans (2015)¹. While pancreatic cancer constitutes 6–7% of these cases (fourth behind lung, breast/prostate and colorectal), only 5–6% of these patients survive 5 years following a diagnosis^{1–2}. The simple, but unfortunate reason for such a high mortality (ranked first across all major

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cancers) is pancreatic cancer's pathophysiology — what causes pancreatic cancer and how exactly the disease manifests and presents in the human body is still, by expert accounts, relatively unknown^{3–4}. Sadly for most patients, diagnoses are ascertained when the cancer has already reached final stages.

In pathology, the determination of a cancer diagnosis is preceded by a highly specific and historically established chain of events, which over decades has been optimized and streamlined for patient care. In brief, the workflow consists of the following steps in figure 1 (text in red).

Between biopsy procurement and visualization, the processes followed by a pathology laboratory represent a distinct, organic blend of automation, general day-to-day procedures and personal technique. These steps are performed typically in an industrial-scale laboratory, which consists of specially trained personnel, large quantities of chemical supplies and reagents, and a significant physical footprint. Ultimately, the process steps are based on three important constraints:

1. biopsy assessment is achieved by *observing specimens in 2D* (i.e., on glass slides) using a bright field microscope.
2. because of 1, tissue biopsies must be physically *sectioned into thin slices*.
3. because of 2, all related physical, chemical and biological procedures have been *developed for a thin section geometry adhered to a glass slide*.

1.2. Microfluidics: “pathology-in-a-tube”

To this end and for the first time, our research team in the Human Photonics Laboratory (HPL) has developed a simple, microfluidic prototype that performs all of the rudimentary steps (figure 1, shaded gray) associated with a pathology laboratory on a prototype, credit card-sized device (figure 2)^{5–6}.

However, histopathological processing on large, unsectioned tissue core biopsies (CBs) precludes the use of simple (2D) microscopy (see constraints above). Fortunately and over the last two decades, a variety of 3D imaging platforms have been developed^{7–18}; their cost and general accessibility have additionally promoted their regular use in many research-based settings. One such system invented and co-developed in HPL is the optical projection tomographic microscope (OPTM)^{16–18}.

1.3. 3D optical imaging: the optical projection tomographic microscope (OPTM)

In brief, the OPTM is a standard inverted microscope that is similar to what is currently used by pathologists. 3D imaging is accomplished by placing biological specimens in glass capillaries. Here, a single, gray scale projected image is acquired after the microscope objective's focus plane is swept across the diameter of the capillary. One projection is thus captured for an incremental angle as the capillary is rotated 360° about its central axis. At 0.72° increments, 500 such projections are processed using MATLAB-based algorithms which render a 3D visualization and produce an *in silico* reconstruction of the biological

specimen. Computer reconstructions permit detailed inspection of patient specimens at any perspective, or angle by a pathologist (figure 3)^{19–22}.

Because biopsy inspection, evaluation and assessment rely heavily on the observation of a specimen, we hypothesize that 3D visualization and reconstruction of tissue CBs may significantly aid pathologists in cancer diagnosis and potentially enhance early detection. Visualization and reconstruction will provide pathologists access to *intact & continuous* tissue architecture in 3D, *in vivo* morphology, cancer tissue spatial distribution, etc. 3D datasets will also aid pathologists within the current, established workflow by providing a general map for a series of traditionally processed microscope slides — valuable tissue “in between” processed slides is inherently lost during sectioning, therefore 3D datasets can recover this information and provide a fundamental gain in the optical information content of a traditionally processed biopsy.

1.4. Integration and pathology

Combined, our prototype microfluidic device and 3D optical microscope form the basis of a proposed rapid on-site evaluation (ROSE) system, which may be seamlessly integrated into the pathology workflow (figure 4). *We therefore envision a system which (before samples are sent to traditional pathology) would process whole, intact tissue CBs that were immediately and directly procured from the patient by a clinician. CBs would then be rapidly imaged in 3D within a time span of minutes instead of hours as in the current paradigm.* If 3D imaging of processed tissue is not possible, or if 3D imaging fails to yield adequate 3D reconstructions due to the overall patient specimen quality, samples may be immediately sent to traditional pathology (figure 4, dark and light green dashed lines). Thus in operation, our system will maximize the information and diagnostic content of patient specimens through nondestructive interrogation with little to no loss in patient sample, cost and time.

1.5. Interface & transport problem space

Nondestructive interrogation however relies on *two significant issues*: 1) physical transfer and 2) transport of tissue while preserving the CB’s mechanical integrity (i.e., “biospecimen preservation”). Importantly, both processes have the biopsy contained in a channel that is sized to be close-fitting, which protects and preserves the tissue structure and CB. In clinical practice, the sizes, geometries and volumes of procured CBs are uncontrollable and are completely dependent on the patient, the type of surgery and clinician technique. In contrast, clinically-employed coring needles (CNs)^{21,22,24} are standardized and permit the design of a microfluidic device to accommodate the minimum and maximum physical parameters of tissue CBs.

Clinical CNs are generally grouped into two types: end-cut and tru-cut²⁴. Using the circumferential edge of the needle end for cutting, end-cut CNs obtain CBs through a method that is similar to a “cookie-cutting” process. In this case, tissue deposition into our microfluidic device requires nothing more than inserting the needle into the main microfluidic channel and using a plunger to propel the CN’s contents into our device. Side-cutting, or “tru-cut” coring needles in comparison procure tissue cores through a side-cutting

process (figure 5). For these needles, the tissue deposition into a microfluidic channel is more challenging since access to the specimen can be blocked by the microchannel's inner wall.

In a preliminary study on general fluid flow and transport⁵, CBs produced by simple needle blunts and a custom-designed artificial coring instrument comprised of microtome blades²³ were deposited into both glass- and PDMS-based microfluidic channels. The corresponding experiments provided the foundation for a more systematic investigation, where precisely measured, well-characterized metal slugs (simulating CBs) were deposited into microchannels in order to understand their impact on laminar fluid flow dynamics (manuscript to be submitted). However, in comparison to metal cylinders, biological tissue contributes significant variability (table 1).

1.6. Study aim

In order to bring our system closer to clinical application, tru-cut clinical coring needles were obtained and used to procure tissue core biopsies from unfixed and fixed porcine and normal/cancerous human pancreas tissue. For the first time, porcine and human pancreas biopsies were deposited into a microfluidic device to study in a first principles approach the transport of tissue within microfluidic channels using controlled, laminar fluid flow. A custom interface was designed and fabricated to facilitate the deposition of tissue core biopsies into the device, and was additionally modeled and simulated to characterize the surrounding fluid flow environment. To ensure that biospecimen integrity was not compromised for subsequent pathology, a novel optomechanical metric was devised to measure and monitor procured, deposited and transported tissue CBs at all stages of the experimental study.

2. METHODS

2.1. Clinical coring needles & tissue specimens

Due to procurement restrictions, only one type of tru-cut clinical CN (Bard® Monopty® 22 mm Disposable Core Biopsy Instrument) was obtained. A total of 10 CN guns were therefore purchased and used in experimentation. Although needle guns are straightforward in use, clinicians (University of Washington Medical Center) were consulted on the proper technique to use such CNs so identical clinical procedures could be replicated at the experimental benchtop.

Porcine pancreas tissue purchased in bulk (Kapowsin Meats, Inc., Graham, WA) was manually cleaned and dissected. Tissue was left unfixed (fresh), or was chemically fixed with standard formalin (10% Formalin w/v, Fisher Scientific) employed in pathology. Tissue was sectioned into large 3–5 cm³ cubes and needle guns were used on such cubes to obtain whole, intact tissue CBs. Needle guns were also employed to obtain CBs from unfixed and fixed normal/cancerous de-identified human pancreas tissue (NWBioTrust, Fred Hutchinson Cancer Research Center/University of Washington/Seattle Children's Hospital).

All experiments with porcine/human samples were conducted under strict protocols in accordance with the policies and standards set forth by the University of Washington Office of Environmental, Health & Safety (UW EH&S).

2.2. Tru-cut needle interface

An interface for a tru-cut coring needle was fabricated based on empirical observations and trial-and-error experimental engineering design. Subsequently, our team determined that the tru-cut needle/microfluidic device interface must be designed to account for the following criteria:

1. *acceptance*: the interface must accept any type of tru-cut coring needle and account for human handling (error).
2. *isolation*: because of 1, the interface must isolate specimens and reduce (or eliminate altogether) specimen contamination and reverse contamination for clinicians.
3. *disposable*: the design must be sterilizable and/or disposable for clinical application.
4. *simple*: because of 3, the interface design must be simple (i.e., no excessive moving parts) and effective.
5. *minimization*: because of 4, the design must transfer tissue into the microfluidic device while preventing, or minimizing, any mechanical damage to the patient specimen.

2.3. Tissue transport experiments

2.3.1. Microfluidic channels—Microfluidic channels ($L = 4.5$ cm, $D = 1.38$ mm) in multiples of four were produced within a single PDMS substrate so that measurements were independent of channel fabrication (figure 7). The cover of an Apple iPod Shuffle[®] container was used due to its size, height and level surface. Four pairs of holes along parallel walls of the container were drilled through and fitted with solid metal rods (figure 7-1). Silicone elastomer (Sylgard[®] 182 Silicone Elastomer Kit, Dow Corning, Inc.) combined with its polymerizing agent in a volumetric ratio of 6:1 (silicone elastomer:initiator) was then poured into the container. Bubbles were removed by placing the container in a vacuum chamber and the entire assembly was finally placed into a 60–70°C oven.

One distinct aspect of producing these microchannels is the method itself (manuscript to be submitted). Open to the oven atmosphere, the top surface of the curing PDMS is exposed to heat while the metal rods conduct heat and simultaneously cure the setting elastomer from within. As a result, 4–5 hr were required to produce a single, disposable substrate with four microfluidic channels.

Substrates were released by running forceps along the boundary of the cured PDMS block while squirting 75% ethanol at the interface. At low viscosity, ethanol wetted the interfaces between the metal molds and the inner microchannel walls and permitted the rods to be slid out with minimal effort.

Microchannels were then placed atop an illumination platform (figure 7-2, 7-3a) beneath a CMOS camera (USB 2.0 Machine Vision Camera, Edmund Optics, Inc.) fitted with several fixed focal length lenses (Edmund Optics, Inc.) (figure 7-3b). The imaging system provided a spatial resolution of 15–50 $\mu\text{m}/\text{px}$ and movies were recorded at 20 fps. Movies began recording after they were electronically triggered by the syringe pump's external serial port (figure 7-3c).

CBs were deposited at the entrance of a microchannel (figure 7-2) using the developed interface, or manually using forceps. Although the connecting Teflon[®] tube was flush with the microchannel inner diameter, CBs were positioned 2–3 mm from the entrance so fluid flow was fully developed after it transitioned through this connection point^{25–26}.

2.3.2. Syringe pump protocol—Ideally, an experiment would consist of observing CB transport at a flow rate for a given amount of time and then increasing that flow rate at an incremental value. The same experiment would then be repeated for the same biopsy a number of times before a new biopsy was used. In practice, we are limited by 1) the water solution volume (2×60 mL syringes), 2) the number of flow rates that can be programmed into the onboard syringe pump computer, 3) the actual range of flow rates in which transport may occur and 4) the fact that samples may be tested only once since biospecimens and the disposable substrate must be appropriately discarded due to biological contamination (UW EH&S guidelines). Consequently, a pump protocol was carefully determined to account for all of these restrictions. For each CB sample, a flow rate was held for a period of 10 s and was subsequently incremented in 0.2 mL/min steps from 0 to 8 mL/min. Each experimental run thus yielded a 6 min 50 s video for each CB sample. Movies were stopped when the CB traversed the entire microfluidic channel distance, or if the pump program reached its end (i.e., 8 mL/min).

2.3.3. “Infinite sink”—Luer-lock syringes with 1.2 cm long, 18G needle blunts (ID = 0.838 mm) were connected to 7.7 cm long Teflon[®] tubes (ID = 1.27 mm) that were fed into a 2-to-1 manifold (Perfusion Manifold, Warner Instruments, Inc.). This manifold was followed by another Teflon[®] tube (L = 2.8 cm, ID = 1.27 mm) that was directly connected to the PDMS-based microfluidic channel. An additional Teflon[®] tube (of the same dimensions) was attached to the corresponding end of this microfluidic channel and inserted into a very long chamber (“infinite sink”) so that the tube end was flush with the chamber's bottom surface (figure 7-3d, figure 8). All constituent components were positioned at this height. This feature and the infinite sink had several important benefits:

1. All components were at equal pressure, which minimized the contribution of any spurious pressure differences between constituent sections of the flow line during the application of constant fluid flow to determine CB transport.
2. Exiting fluid flowed into a large surface area shallow pool, which was held together by surface tension. In this fashion, a continuous fluid medium was maintained. If fluid simply emptied into a beaker, the action of discretely exiting drops would affect the entire flow line and hence CB transport.

3. Water surface tension and capillary action permitted the shallow pool to grow by spreading. Spreading kept the pool depth at constant height, which also equaled the connecting Teflon[®] tube diameter. If the bath was allowed to grow in height, an opposing and inconsistent hydrostatic pressure would retard an applied fluid flow. This was inadvertently discovered during early experimentation.

2.4. Optomechanical metric: CB integrity

A novel optomechanical metric was devised as a means to monitor CB mechanical integrity. Since CBs are being transferred from the CN to the microfluidic device (and eventually from the device to the OPTM, or pathology), such monitoring is required to ensure sample viability downstream for pathology. Using the artificial coring instrument (see **Introduction, Interface & transport problem space**), porcine pancreas tissue CBs were produced and precisely measured using a high resolution digital camera. Samples were placed on the illumination platform and a line of intensity was measured along the central axes of these specimens. Data were averaged and a multiple of the standard deviation was determined at which mechanical breaks could be assessed, or detected with a given CB. The algorithm was then used on clinical CN-derived CBs prior to device insertion and following transport experimentation in order to assess CB mechanical integrity.

3. RESULTS

3.1. Clinical coring needles & tissue specimens

Many unfixed and fixed porcine/human CBs were obtained using the clinical CN gun during practice sessions. For formal experimentation, a running tally out of 15–20 tries was recorded as a qualitative and semi-quantitative first step to assess procurement and transport efficiency (table 2).

Unfortunately, no unfixed (fresh) porcine pancreas CBs were able to be obtained for the above experiment. At a later time, when formal experiments were already concluded, unfixed porcine CBs were readily obtained from a new porcine tissue source. We suspected that the earlier porcine pancreas tissue provided by the supplier may have already undergone significant tissue degradation and autolysis. When using the tru-cut CN gun, unfixed and fixed porcine tissue yielded CBs with the following dimensions in millimeters:

clinical needle	porcine	porcine
CB compartment	unfixed (n = 30)	fixed (n = 30)
L = 17	L = $5.7 \pm 29\%$	L = $6.0 \pm 28\%$
D = 0.350–0.360	D = $0.4 \pm 28\%$	D = $0.425 \pm 20\%$

3.2. Tru-cut needle interface

Several materials were proposed for the clinical CN/microfluidic device interface, such as glass, paraffin and acrylic, but designs ultimately settled on using the same material as the original microfluidic device (i.e., PDMS). PDMS is sterilizable, disposable and optically clear, which permitted direct observation of the transfer of tissue CBs.

Initially, the interface was designed as two separate pieces which were bonded together. However, improper alignment and inconsistent performance ruled in favor of a seamless design fabricated from a single, simple PDMS substrate. Cross sectional geometries (i.e., star-, square- and threaded screw-patterns) were also explored for the chambers within this substrate that were designed to handle the tru-cut needle, but a funnel geometry provided the optimal balance for the proposed design constraints.

Therefore, an inline funnel-reverse funnel design (figure 9-1) was decided upon and fabricated in a fashion consistent with the development of the microfluidic channels. The interface was created by using micropipette tips that were centered using metal rods (figure 9-2). A sharply angled rod was positioned so it intersected one of these micropipette tips circumferentially and at half its length. After PDMS curing, the container and skeletal mold were removed. The reverse funnel with the intersecting rod (i.e., the mold for a “loosening microchannel”) was called the “loosening” zone, while the corresponding funnel was called the “entry” zone (figure 9-3). Following mold removal, a new micropipette tip (figure 9-3, in yellow on left) was inserted into the empty funnel of the entry zone. The micropipette tip served as a cheap, sterilizable and disposable smooth shell, which permitted gentle funneling of the tru-cut CN even with jostling, or sudden jerking movements associated with human handling, or error. The shell was also hard, which additionally prevented the user from accidentally inserting the CN into the surrounding soft PDMS substrate.

Residing between the funnels was a small gap that was filled-in with PDMS. This stop gap combated contamination and reverse contamination by providing a press-fit, one-way entrance for inserted CNs (figure 9-1, 9-3). Once inserted, the CN could not be accidentally pulled out as the press-fit, sealed entrance would catch the side-cutting geometry of the needle and prevent needle removal.

Once a CN was finally inserted into the interface, the unsheathed tip was automatically positioned within the loosening zone. A needle, or flexible tube was inserted into the loosening microchannel and fluid flow was then used to loosen the tissue CB from the unsheathed CN. Because the loosening zone is a reverse funnel, there is a significant, but graded reduction in hydraulic resistance which promotes CB loosening.

The reverse funnel geometry of the loosening zone was also smooth. This aspect allowed the back end of a smaller 10 μ L micropipette tip to be inserted into the funnel, while the front (sharp) end was inserted into the main channel of the microfluidic device (figure 9-4). Gentle fluid flow thus propelled the dislodged CB from the loosening zone and into the main microfluidic channel for subsequent experimentation with no moving parts and with little damage to the patient specimen. Therefore all design criteria (“disposable”, “simple”, “acceptance”, “isolation” and “minimization” — presented above in that order) were satisfied with the developed interface.

Fluid flow in the loosening zone (figure 9-4, red rectangle) to dislodge CBs is a complex process and is dependent on many factors. At the very least, CB dislodgement is a function of the funnel geometry, the position of the loosening microchannel and the distance the clinical CN was inserted into the zone. Therefore, a basic ANSYS simulation with the CN at

two inserted excursion depths (20 and 7 mm — 90% and 50% of the zone length, respectively) within the loosening zone was developed to understand the local fluid flow and the local fluid environment. In the simulation, the loosening microchannel was not located in the exact position as was fabricated (figure 10, pink dotted arrow) due to software constraints, so the source of fluid flow was placed at the location of the stop gap (figure 10, pink solid arrow). This configuration was not so different from transport experiments, where CBs positioned at the entrance of microchannels were exposed to various fluid flow rates. Simulation input flow rates of 3, 7 and 15 mL/min were chosen as 50%, 100% and 200% of the maximum flow rate applied in transport experiments on the benchtop. Simulations yielded wall shear stress and pressure data along the simulated tru-cut needle, and velocity fields surrounding the CN.

Velocity field data provided the most relevant results from the computer simulations. For reference, applied experimental flow rates (0.2–8.0 mL/min) within empty microfluidic channels correspond to average fluid velocities of 2–90 mm/s. At 15 mL/min (200% of the maximum flow rate applied in experiments), velocity fields about simulated CNs ranged in magnitude from 0.3–0.7 $\mu\text{m/s}$. Their field distributions also extended approximately 7 mm from the source of the fluid flow.

3.3. Tissue transport experiments

Tissue CBs successfully deposited within microfluidic channels were exposed to fluid flow rates from 0 to 8 mL/min in 0.2 mL/min increments to determine the threshold for CB transport. Movie recordings of experiments were analyzed in a systematic approach (figure 11). Recordings were first converted to individual image frames and inverted. The contrast was adjusted to saturation such that CBs and the background transmissive lighting appeared white and black, respectively (figure 11-1). Each image row was then collectively summed to produce a single intensity profile for the entire image (figure 11-2). Because of the variability of CB size, shape and geometry, an optical center of mass (COM) was calculated for each intensity profile as an accurate measure of CB position within the microfluidic channel (figure 11-2, dashed vertical line in red). The entire experiment (movie) produced a position versus time record for the CB as it translated across the microfluidic channel under various flow rates (figure 11-3).

A high degree of variability is present when considering biological tissue transport on a microfluidics platform (see **Introduction**, Table 1). The position record over time in figure 11-3 was a common, representative example across all specimens: CBs sometimes suddenly moved at one flow rate and then at various other flow rates crept slowly across the length of the microfluidic channel. In other cases, the experiment was all-or-nothing: CBs remained stationary until a critical flow rate was reached, at which point the tissue biopsy suddenly translated across the entire channel length. And in a significant number of cases, CBs transported a short distance (less than the total microchannel length) and remained stationary until the pump program concluded. To this end and as a first approach, only the minimum transport flow rate was determined for each experiment.

The minimum transport flow rate data at which CBs began to translate across the microchannel length were measured (figure 11-3) regardless if the tissue biopsies traversed

the entire microchannel length within the given pump program time. Data were recorded and averaged for each group of tissue specimens. Due to the high variability associated with biological tissue and limited range/resolution of the pump program, error of the averaged data reached 100%, but never exceeded 130%. Consequently, the minimum, mean and maximum values for the transport flow rate of tissue CBs are reported in figure 12.

3.4. Optomechanical metric: CB integrity

CB transfer via a custom interface and/or CB transport using microfluidic channels require a basic metric to monitor mechanical integrity to ensure sample viability and biospecimen preservation for subsequent pathologic analysis. Averaged measurements (see **Results, Clinical coring needles & tissue specimens**) of CN-derived CBs yielded an approximate 30% error in diameter. This result demonstrated that clinical CNs produced CBs of a relative consistent thickness. Consequently, we assumed transmissive lighting intensity and photon scattering would be similar across independently measured CBs. Thus a line intensity across porcine pancreatic CBs produced by the artificial coring instrument was averaged (I_{avg}) and a standard deviation (σ) was measured. We determined that when intensity profile data deviated beyond the $I_{avg} + 2\sigma$ boundary that a CB was mechanically compromised (i.e., at that position, a tissue CB was physically broken) (figure 13).

Sensitivity and specificity of the metric were determined on both training CB sets and experimental (CN-derived) ones. By randomizing CB images, a user would visually determine if there was a physical break in the tissue CB. If the optomechanical metric detected such a break, this trial was considered a “true positive”. A similar set of criteria were followed for “false positive”, “true negative” and “false negative”. Once complete, it was determined that the optomechanical metric consisted of a sensitivity of 88% and a specificity of 79%. When CBs derived from CNs were used, metric sensitivity and specificity were 100% and 50%, respectively.

4. DISCUSSION

4.1. Clinical coring needles & tissue specimens

A 67% success rate for procuring CBs using clinical CN was more than adequate for benchtop research. The success rate would have been higher, but was primarily due to the lack of successful procurements with degraded unfixed (fresh) porcine pancreas (see **Results, Clinical coring needles & tissue specimens**). Success rate was also dependent on the fact that needle guns were sometimes misfired due to user mishandling and that tissue was not always procured as a whole, intact continuous piece, but disparate tissue chunks. Although human samples were limited, there were no obvious qualitative differences in procuring tissue CBs when comparing normal and cancerous tissue.

Because of the inherent variability of clinically-procured tissue, we devised a simple qualitative/semi-quantitative method to assess procurement and transport efficiency for a clinical CN gun. Based on tabulated trials, we were able to obtain CBs with a 67% success rate. Of these samples, 66% were partially, or completely transported across the length of the microfluidic channel under the pump protocol. In future work, partially transported (or all) CBs may easily traverse the entire microchannel distance by pumping fluid in quick

succession (i.e., impulse of fluid flow, pulsatile flow, etc.). Care must therefore be taken to ensure that CBs are not mechanically damaged during these pumping patterns.

Regardless of fixation, CNs produced similar length, similar diameter CBs with similar error in measurement (~30%). These data and data from future CNs will play a role in the microchannel dimensions within future designs and iterations of our microfluidic device. CB diameters (thicknesses) will also help refine a “real-time” optomechanical metric for quality control such that specimens are viable and preserved for downstream pathology.

4.2. Tru-cut needle interface

The custom interface satisfied the engineering design constraints proposed by our team and successfully deposited CBs within our microfluidic device. While we expected significant CB damage during insertion through the stop gap, or failure to dislodge the CB sample within the loosening zone, instead we encountered an unexpected scenario. In several cases, the interface could not prevent already compromised CBs from doubling-over and folding in-half as they were deposited within the main microfluidic channel (figure 14).

Simulation results of the fluid velocity field within the loosening zone were somewhat intuitive. We expected velocity fields to be greater in magnitude at the source (i.e., the stop gap) and to linearly decrease over the length of the inserted needle's shaft. Despite a simulated flow rate (15 mL/min) that was twice the maximum for transport experiments (8 mL/min), we did not expect the velocity field magnitudes to be so much lower than velocity values corresponding to CB transport within our microchannels. Therefore, if CB dislodgement requires fluid flow velocities similar to transport experiments, then it stands that the input source flow rate must be significantly greater than 15 mL/min. While it is an assured fact that the loosening microchannel location and the funnel geometry greatly impact the analysis, this first order finding is nonetheless still relevant since the flow rate to dislodge CBs was not controlled and instead was accomplished by trial-and-error using a hand pumping device.

4.3. Tissue transport experimental setup

Higher fluid flow rates were required for unfixed and fixed human samples, respectively, while a lower flow rate was required for fixed pancreas tissue CBs. Pancreas CBs were procured from large tissue blocks that were thoroughly cleaned and at best isotropic and lacking internal crevices. Therefore these CBs were very smooth and mechanically continuous. On the other hand, human samples were rough and displayed kinks in the CB structure due to sample availability and pre-handling by clinicians. To this end, lower flow rates were required for streamlined (porcine) tissue CBs than irregularly shaped (human) ones.

Fixed tissue is denser than unfixed tissue, thus we conclude unfixed human samples were easier to transport than fixed CBs since fixed samples rested on the bottom of the microfluidic channels during which surface forces may have impeded CB transport (see **Introduction**, Table 1). Mean transport flow rate and range (minimum to maximum)

increased across tissue groups such that from fixed porcine to fixed human samples, the transport flow rate mean and range increased by 10x and 15x, respectively.

4.4. Optomechanical metric: CB integrity

CN gun procured tissue CBs were measured using the devised optomechanical metric before and after CB transfer and transport experiments. The metric confirmed that the interface and transport experiments preserved the mechanical integrity of CN-derived CBs. However, we discovered that 73% of all CBs were mechanically compromised and were due to the CN itself not through our intervention. Based on our metric, we would have normally sent these samples immediately to traditional pathology instead of processing and/or imaging them within our hybrid system.

During training sessions, our optomechanical metric displayed a sensitivity of 87% and a specificity of 79%. When the metric was used on clinically-relevant CN-derived CBs, sensitivity reached 100% and specificity dropped to 50%. Our metric thus accurately assessed when a CB was in separate pieces, or was mechanically compromised. However, a lower specificity was due to the viscoelastic properties of tissue. When two separate tissue pieces were connected by a thin membrane, the CB by our metric was deemed compromised — optically, transmissive light could pass through the membrane as if no tissue was present even though the entire biopsy was mechanically continuous when directly inspected by eye.

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Figure 1.

Workflow of cancer diagnosis in pathology. The total turnaround time from patient procurement to diagnosis by a pathologist can vary from 24–72 hr. Using microfluidic technology in conjunction with 3D optical imaging, all the rudimentary steps of a pathology laboratory (shaded gray) have been recently consolidated into a prototype, credit card-sized device to promote rapid biopsy processing and point-of-care diagnostics^{5–6}.

Figure 2.

Basic pathology within a microfluidics circuit shown over a time course of less than 3 min (right top to bottom). A porcine pancreas tissue CB was inserted using a coring needle into the main microfluidic channel of our microfluidic device. There, it was chemically fixed and then positioned within the center of a “U-channel”. We have proposed that such U-channels may populate either side of the main microchannel like gas stations on a highway. Each “U-station” would then be responsible for a particular step in the pathology workflow. Above, the CB was stained with standard hematoxylin employed in pathology, washed and then transported using laminar fluid flow. To the best of our knowledge, this is the first time that processing and/or transport of large tissue has been demonstrated in a microfluidic system^{5–6}.

Figure 3.

3D visualization and reconstruction of a dense, fine needle aspirate (FNA), or (simulated) sparse, optically cleared tissue CB. Prior to imaging large tissue CBs, gel suspensions seeded with a high concentration of human lung cells (normal and cancerous) were imaged as an initial proof-of-concept. Cultured cells were processed (i.e., fixed, stained and cleared) in a fashion consistent with pathology. Therefore these samples were identical to FNA tissue biopsies which would be procured from lung cancer patients, but were significantly denser^{19–20}. (1) Red box encloses a section of the capillary tube filled with processed lung cells as would be viewed at 40x using the eyepieces of a microscope. After capturing 500 projections at this location, the tube was translated so corresponding capillary sections could be imaged. (2) Simple stitch algorithms²³ generated a panoramic picture for each incremental angle of rotation (here, $\theta = 0^\circ$). Due to a lighting artifact, an optical “crease” was seen where corresponding images were aligned, registered and stitched together. (3) 3D visualization of the FNA. A cancerous and normal lung cell (peanut-shaped and circular outline in blue, respectively) were visually tracked at 90° , 180° and 270° . (4) The specific capillary section (1) enclosed in red, which consisted of 500 projections and contained the (3) tracked cancer cell, was (5) reconstructed. The blue arrow points to the cancer cell of interest. The orange arrow points to the underbelly of the cell, which is not viewable at this angle ($\theta = 0^\circ$), nor was it viewable at any other angle since other suspended cells block a direct line-of-sight. Through reconstruction, the optical dataset was (6) translated, rotated and magnified so the cancer cell’s underside was optically accessible for pathologist evaluation and diagnosis.

Figure 4.

Microfluidic-OPTM integration into pathology. Our hybrid microfluidic-OPTM system may provide (3) an alternative, but additive pathway to (1–2) traditional pathology. Aimed for performing rapid evaluation in 10–20 min, or less, the system contains (4–5) check points where the preserved CB may be instantly removed and sent to traditional processing if so desired by the pathologist. However, while tissue processing (i.e., microfluidics) and 3D imaging (i.e., OPTM) have been separately demonstrated, total system functionality and integration relies upon physical interfaces between constituent processes and components (i.e., clinical coring needle to microfluidic device), and the controllable transport of tissue CBs within microfluidic channels (i.e., CB transport from device to OPTM, transport from device/OPTM to traditional pathology).

Figure 5.

Spring-action tru-cut clinical CN gun (Bard® Monopty® 22 mm). The gun is (1) hand-held and the (2) CN is covered by a (3) sheath which is retracted away during initial loading. The compartment for the CB here is 17 mm long and 0.35–0.36 mm in diameter. After placing the coring needle/sheath (4) under spring-tension, the (1) trigger may be depressed to insert the coring needle into a (5) tissue block. Occurring extremely fast, (5) the coring needle advances followed by the rapid succession of the sheath, which cuts out a CB. When reloading the gun, (6) the sheath is retracted away, which permits access to the tissue CB by a) manual handling, b) immersing the compartment into a volume filled with a chemical fixative, or c) employing fluid flow through an interface (the current study).

Figure 6.

Representative examples of the experimental variability for CBs. (a) Fixed human pancreas CB initially appeared as a small tissue chunk within the PDMS microfluidic channel ($D = 1.38$ mm, $L \sim 50$ mm). During transport, the CB unexpectedly unraveled (blue arrows). Although the (b) fixed porcine pancreas CB was transported at the same flow rate as (a) the previous biopsy, the difference in the distance traversed is a function of CB size, geometry, and the innerspace between the CB and microchannel inner wall. (a, b, d) The contrast of all the images captured from movie records was adjusted to better visualize CBs. Under (c) nominal experimental conditions, unfixed (fresh) human pancreas CBs were difficult to visualize (red arrow). Even when contrast was adjusted, these CBs either disappeared by matching the transmissive background lighting, or microchannels appeared so grainy that CB tracking within the movie was nearly impossible. In comparison to (b) fixed porcine pancreas CB, (d) fixed human pancreas CBs exhibited a less “smooth” geometry due to sample availability — porcine CBs were obtained from large, well-cleaned, isotropic tissue blocks whereas human CBs were procured from 2–3 mm thick postage stamp-sized samples that were pre-handled by clinicians who required subsamples for ancillary diagnostic testing. All CBs here were obtained with the same CN and technique.

Figure 7.

Experimental setup for tissue CB transport. (1) Metal rods served as a skeletal mold within a plastic container (channel length = 4.5 cm). Curing PDMS was poured into the container and allowed to set. An alcohol releasing agent allowed the removal of the container and metal rods using forceps and pliers with ease. The microfluidic channel-containing substrate was then placed on (2) an illumination platform in order to record movies of CB transport. (3) The entire experimental setup consisted of a syringe pump, 60 mL syringes, connecting Teflon® tubing, a microfluidic channel and sink. Movie recordings were captured by a CMOS camera and lens, and individual recordings (experiments) were acquired and analyzed using custom written programs in LabView (National Instruments, Inc.), ImageJ (NIH) and MATLAB (Mathworks, Inc.).

Figure 8.

The “infinite sink”. By keeping all constituent components of the experimental setup at the same height, pressure development across any section of the flow line was a function of constant current (i.e., fluid flow) and its specific hydraulic resistance (i.e., $P_{\text{sect}} = Q_{\text{const}} R_{\text{H, sect}}$). Fluid flow and pressure are directly impacted when fluid exits the system discretely (i.e., drops in a beaker), at a much lower pressure (i.e., tube placed in a filled beaker below the benchtop surface), or in a narrow chamber (i.e., a chamber where the bath volume grows in height over time, which develops a significant and opposing hydrostatic pressure to the exiting fluid flow). With a long sink, the shallow water pool (at the same height as the connecting Teflon® tube) can spread (increase volume) while preventing the development of an opposing hydrostatic pressure.

Figure 9.

Tru-cut needle interface design and fabrication. The design of the interface first began as (1) a SolidWorks model and was (2) then fabricated using the cover of a microscope slide holder, two 200 μL laboratory micropipette tips and metal rods used earlier for microfluidic channels. The sharp ends of the micropipette tips were cutoff to promote a press-fit connection with an inserted tru-cut needle. (3) After removing the skeletal mold, a new 200 μL tip was placed in the entry zone funnel. (4) A magnified image of the loosening zone. The back and sharp end of a smaller 10 μL micropipette tip were cut-off. This permitted the tip to be a) press-fitted into the funnel of the loosening zone and b) inserted directly into the main channel of our microfluidic device.

Figure 10.

ANSYS simulation of inserted clinical CNs at two insertion depths within the loosening zone. Due to software constraints, only axisymmetric simulations were permitted, therefore the images here are lengthwise cross sectional slices along the axis of the loosening zone. Pink dotted arrow represents the position of the loosening microchannel (and source of the loosening fluid flow) in the actual interface. Solid pink arrow represents the source of the fluid flow within the simulation. Colored distributions are the resultant velocity fields in micrometers per second following successful runs of the simulation. The white “graininess” of the velocity field distribution in the top image is a consequence of simulation meshing and was an artifact of image capture.

Figure 11.

Tissue CB transport analysis. (1–2) Movie recordings were saved as individual image frames that were inverted. Contrast was adjusted such that when the image rows were summed widthwise, the resultant intensity profile illustrated the lengthwise boundaries of the CB. An optical COM was calculated for each intensity profile (image frame) and (3) tracked over the time course of the movie recording. (3) Each gray vertical line within the plot represents a time point when the flow rate was incremented by 0.2 mL/min. The minimum transport flow rate was visually determined from the COM trace for a given experiment.

Figure 12.

The minimum, mean and maximum values of the minimum transport flow rate dataset across all specimens tested.

Figure 13.

Optomechanical metric applied to a training (set I) and experimental (set II) dataset. Set I: porcine pancreas CBs procured by the artificial coring instrument (not the clinical CN) and their corresponding intensity profiles. Red rectangles denote visually-confirmed mechanical breaks in the CB. Orange rectangle indicates a case where a break was detected by the novel metric, but was false due to lighting and edge effects of the CB. Green rectangle demonstrated a possible physical break, but was not detected by the metric (rare case). Blue rectangle illustrated a break detected by the metric, but visually the CB was still mechanically continuous. Set II: porcine pancreas CBs procured by clinical CNs and their corresponding intensity profiles. For all intensity profiles: solid horizontal red line = I_{avg} , dotted horizontal red line = $I_{\text{avg}} + 2\sigma$.

Figure 14.

Unexpected results using the developed tru-cut needle interface. If CBs were already mechanically compromised, these samples on occasion would fold in-half and remain in that configuration as they were deposited into the main microfluidic channel of the device.

Table 1

Biological considerations (i.e., sources of variability) for transporting tissue CBs within a microfluidic device.

material	density viscoelasticity porosity	<ol style="list-style-type: none"> 1) fresh, or chemically fixed tissue CBs may be neutrally buoyant, or more dense than the surrounding fluid environment. 2) stiffness of fresh, or chemically fixed tissue can range from a gelatinous to a more solid state, hence CBs can stretch and/or compress (figure 6a). 3) tissue is porous, thus fluid flow can occur <i>through</i> the entire CB.
macroscopic	size geometry	<ol style="list-style-type: none"> 4) CBs are not perfect cylinders, therefore fluid dynamics along CB boundaries is complex. 5) transport (i.e., static to moving reference frame) is related to the CB-microchannel wall inner space (figure 6b). 6) transport is hinged on geometric constraints (i.e. length <i>and</i> diameter of the CB) (figure 6a, b).
microscopic	surface properties intermolecular forces	<ol style="list-style-type: none"> 7) biological tissue can exhibit both hydrophilicity and hydrophobicity (i.e., glass- and PDMS- based microfluidic channels are hydrophilic and hydrophobic, respectively). 8) tissue CBs and microchannels are rough, which impacts transport through frictional forces. 9) CB-microchannel adhesive forces (i.e., electrostatic, capillary action) may impede transport.
in operation	bubbles optical tracking human tissue	<ol style="list-style-type: none"> 10) temperature and the fluid medium itself produce micro- and nano-bubbles, which are sticky, impede flow and cause inconsistent, nonlinear fluid flow. 11) unfixed tissue in small volumes is nearly optically clear, which makes sample tracking increasingly difficult (figure 6c). 12) procured human samples are variable and depend on the clinician, surgery and specific technique employed (figure 6d).

Table 2

Tissue CB procurement and transport efficiency. Successful procurement = of the coring attempts, number of times whole, intact CBs were procured. Successfully deposited = of successful CB procurements and using 1) the custom-designed interface, or 2) manual methods, number of times tissue was successfully deposited into the microfluidic device. Partial transport = number of deposited CBs which traversed only part of the microchannel after the pump program was complete. Full transport = number of deposited CBs which traversed the entire microchannel length.

	coring attempts	successful procurement	successfully deposited	partial transport	full transport
porcine unfixed	20	5	0	0	0
porcine fixed	15	15	10	1	9
human unfixed (normal/cancer)	20	15	11	4	7
human fixed (normal/cancer)	15	12	10	1	9