UnipicK™

Cell and Tissue Acquisition System with Universal Straddle Attachment

User Manual

2019

Contents

1	Introduction	1
2	Safety	2
3	Components and Accessories	3
4	Initial Set Up	4
5	Using **UnipicK™**	7
6	Sample Protocols	22
7	Troubleshooting	31
8	Technical Specifications	33
9	System Performance	34
10	Warranty and Liability	35
11	Contact	35

1 Introduction

UnipicK™, a capillary-based cell and tissue acquisition system, is the key to reliable, precise, and affordable cell and tissue sample acquisition prerequisite for a range of *in vitro* studies, including cell and region specific tissue experimentation and single cell analysis.

This vacuum-assisted capillary based system is compatible with most inverted microscopes and allows for rapid and efficient acquisition of specific cells from adherent cell cultures based on their morphology, location or fluorescent label. It can also collect single cells from suspension cell cultures and individual multicellular spheres from three-dimensional (3D) cell cultures. **UnipicK™** collects cells without compromising cell viability, thus enabling primary culturing or recultivation of each of the collected cells.

In addition to the collection of individual cells from culture dishes, **UnipicK™** performs isolation of single cells and subanatomical regions from various brain tissue samples prepared by different methods, such as fresh frozen tissue, sucrose treated tissue and fresh live tissues, with minimal contamination of surrounding components, while leaving the intracellular structure and molecules intact. Moreover, it works with thicker tissue sections up to 500 µm, suitable for analyses that require large amounts of sample material, such as proteomics.

Samples collected using **UnipicK™** may be used for a wide range of downstream applications and techniques used in modern molecular biology targeting both protein and nucleic acids, including, but not limited to, quantitative RT-PCR, global gene expression, epigenetic, and proteomics studies.

2 Safety

1. Handle glass capillaries with care. Capillaries have sharp tips and can break on or under skin surface upon contact. It is strongly recommended to always wear goggles and gloves during handling.
2. Install **UnipicK™** over an inverted microscope, level surface and allow at least 5 inches of space all around **UnipicK™** and microscope for ventilation.
3. Always use the power supply and cord provided with the unit. If a proper power supply and cord are not used, product safety performance cannot be warranted.
4. When installing **UnipicK™**, route the power cord away from the microscope frame and **UnipicK™** unit.
5. Always ensure that the grounding terminal of the **UnipicK™** unit and that of the wall outlet are properly connected. If the unit is not grounded, NeuroInDx cannot warrant electrical safety.
6. Never allow metal objects to penetrate any openings on the **UnipicK™** unit as this could result in user injury and damage to the instrument.
7. Do not insert objects into the capillary holder other than filters and disposable capillary units (DCUs).
8. When **UnipicK™** is not in operation, be sure to turn the power off and disconnect the power cord from the connector socket of the **UnipicK™** unit or from the wall power outlet.
9. Dispose used capillaries (DCUs) into a biohazard glass waste container according to regulations.

Caution: UnipicK™ components contain hazardous materials. **UnipicK™** should be disposed according to local regulations.

3 Components and Accessories

UnipicK™ (**Figure 1**) comes equipped with a Universal Straddle that may be fitted over most inverted microscopes, **UnipicK™** unit (Sampler Head at top with adjustable LED ring light, Vacuum Line and Electric Cables, and Control Box with vacuum module), and a power supply with cord. Ready-to-use Disposable Capillary Units (DCUs) for cell collection and tissue dissection and filter attachments are sold separately.

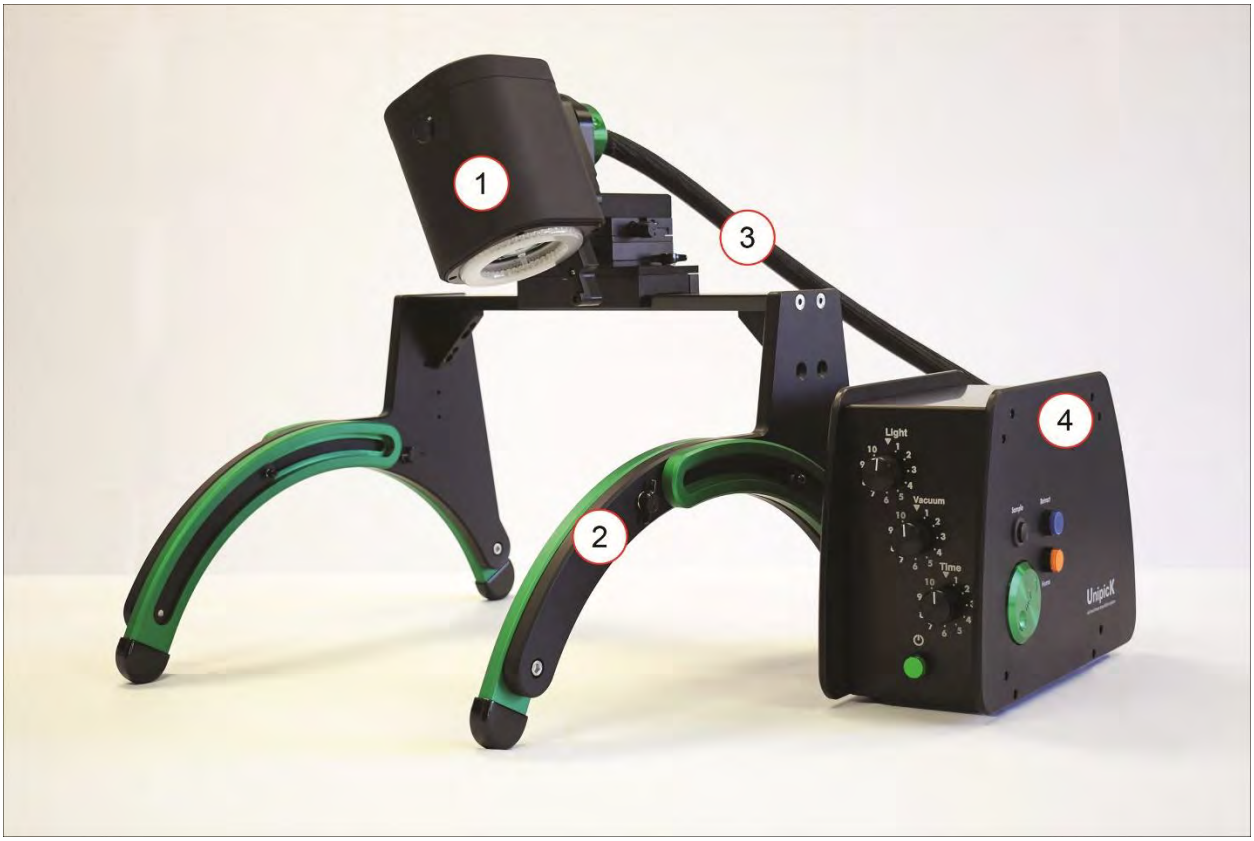


Figure 1: *Major components of **UnipicK™**: (1) **UnipicK™** Sampler Head; (2) Universal Microscope Straddle; (3) Vacuum Line and Electrical Cables; (4) **UnipicK™** Control Box with light intensity dial (top), vacuum strength dial (middle), vacuum impulse duration dial (bottom) and DCU controls.*

4 Initial Set Up

All necessary components are included in the packing. Make sure that the following items have been received upon opening the package.

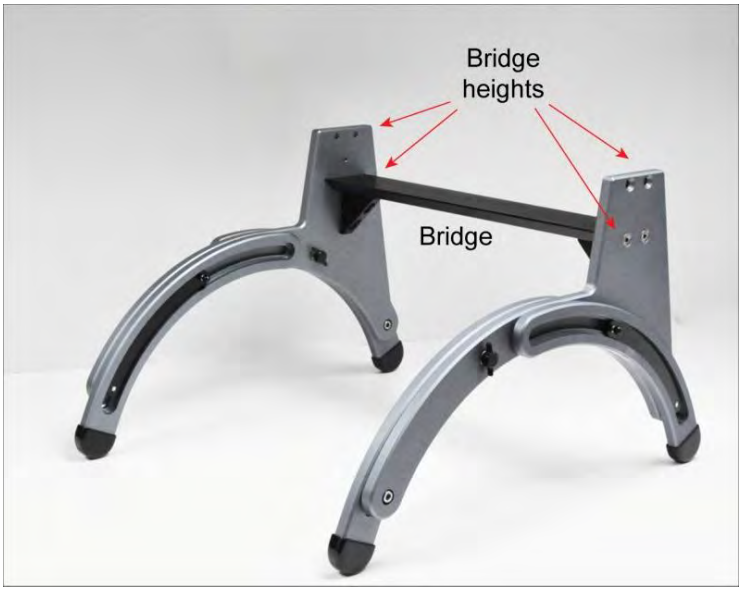
1. **UnipicK™** unit (Head and Control Box) (1)
2. System Alignment Tool (1)
3. Universal Straddle Stand with a bullseye level, a pair of gussets, and short and long horizontal bars (1)
4. Hex wrenches (3, 4, & 5 mm)
5. Power supply and cord (one of each)

Proper installation of all parts is required for **UnipicK™** to work properly. Make sure the following steps are completed before attempting to use the system. Refer to your microscope manual for operation specific to the microscope. **It is recommended that a crosshair reticle be placed in the eyepiece before using UnipicK™.**

NOTE: **Instructional video is also available on our YouTube channel: http://www.youtube.com/NDXInc.**

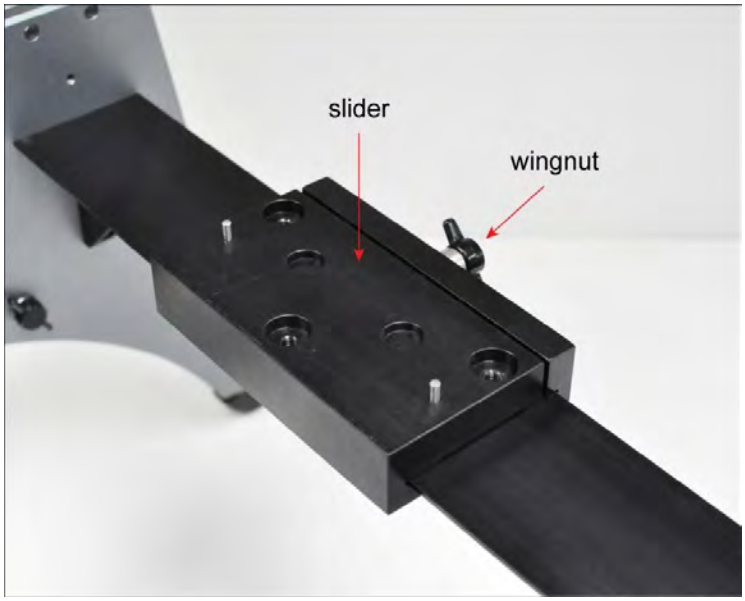
1. Before placing **UnipicK™** over the inverted microscope the phase light must be removed from the microscope. Refer to the microscope manual or contact the microscope manufacturer for assistance.

2. Assemble the Universal Straddle by attaching a bridge (short or long) to the curved legs with a pair of gussets and screws provided with the unit. DO NOT TIGHTEN THE SCREWS AT THIS TIME. There are two possible vertical positions where the bridge can be attached. Choose the appropriate height depending on your microscope.



3. Using a bullseye level (provided) locate a level surface where the instrument will be installed. Place the Universal Straddle on the surface and level by placing the bullseye level on the bridge. Tighten the screws for the gussets and the sides of the curved legs to secure all parts in the leveled position.

4. Loosen the screw on the slider and place it on the center of the bridge with the wingnut facing the back**.** Tighten the screw to secure the slider on the bridge.



5. Attach the System Alignment Tool to the slider and place the Universal Straddle with the Alignment Tool attached over the microscope. If necessary, move the mechanical stage on the microscope to allow the System Alignment Tool to rest directly on the microscope stage.

6. Use one hand to hold the System Alignment Tool in place (**red arrow**). Using the other hand, loosen the screws with wingnuts and slide each of the legs down until they all rest on the benchtop. Once the legs are down on both sides, tighten the screws to secure legs in place.

7. Looking down onto the System Alignment Tool, visually align the center opening on the Tool to the microscope objective lens (**red arrow**).

8. Remove the System Alignment Tool and carefully attach the **UnipicK™** Head to the slider and secure by tightening the screws.

Power supply and cord

Connect the power supply and the cord. Plug the female cord plug into the power jack located in the rear wall of the **UnipicK™** side chassis and plug the power cable into the outlet.

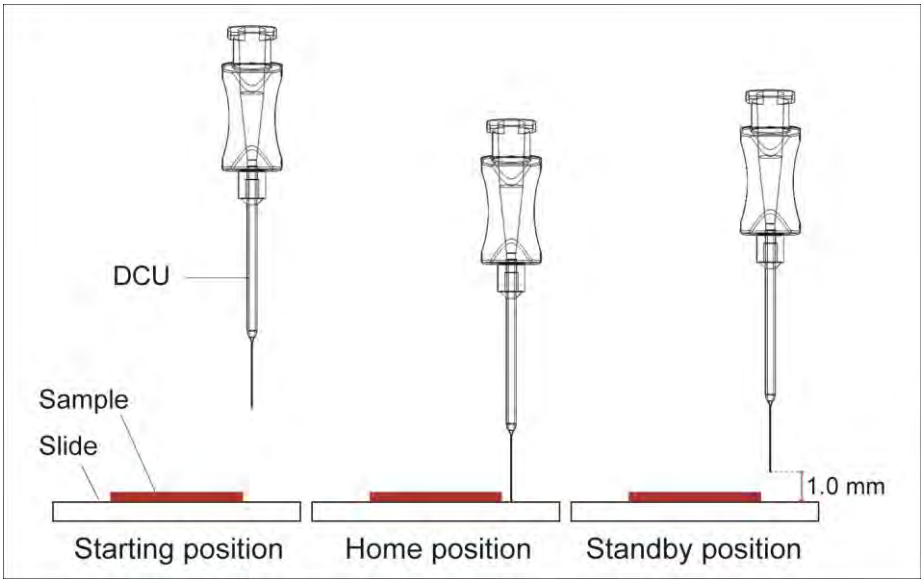
5 Using UnipicK™

Terminology

Starting position: Highest vertical position for the DCU; the initial position when the unit is first turned on and capillary has been mounted or after it is reset.

Home position: Calibration of Collecting/Dissecting position of the DCU tip determined by the user. Homing is performed prior to every collection/dissection procedure.

Standby position: 1 mm above the Home position. DCU will be lifted to Standby position when the orange *Home* button is pressed. DCU returns to Standby position after each sampling until the Home position or the system has been reset.



Power

To turn power on, press the green push button on the front panel of the Control Box. The button will illuminate in depressed position when turned on. Press again to turn unit off (**Figure 2**).

Filter and DCU attachment

Turn the **UnipicK™** Head 90˚ counterclockwise to expose the male luer connector (**Figure 3**). Holding the outer rim of the filter, connect the female luer lock connector of the filter to the male luer connector on the **UnipicK™** Head. Do not overtighten the filter, as this will damage its female luer lock. Next, attach a DCU to the filter.

Caution: DCU consists of two parts, the hub base with a female luer lock connector and a glass capillary component. Always handle a DCU by the hub base to avoid injury and damage to the capillary.

X-Y control

To center the tip of the DCU in the field of view, use the manual dials (**Figure 3**) on the linear stages/micromanipulators at the base of the **UnipicK™** Head where it mounts to the Universal Straddle.

Positioning

To position the tip of the DCU along the z-axis, use the green knob (**Figure 2**) to move up (counterclockwise turn) and down (clockwise turn). A slow small turn will move the DCU by 1.5 µm indicated by a single click sound. When the knob is turned quickly, the DCU will move at a rate of 225µm/s.

Home

Use the green knob to set the Home position for sampling, i.e. the desired position of the DCU's tip on the z-axis for sampling (**Figure 2**). When the capillary tip comes in contact with the tissue surface or cells, press the orange *Home* button (**Figure 2**). When the Home position is designated, the *Home* button will illuminate and DCU will move 1 mm above that position on the z-axis to the Standby position.

Vacuum level and duration control

The two dials at the front of the side chassis (**Figure 2**) control the vacuum level from 2.2''Hg (1) to maximum of 22''Hg (10) and the vacuum duration from 100 ms (1) to maximum of 1 s (10). Depending on the sample and the desired result, various vacuum levels and duration may be used. We recommend testing these parameters prior to any collection/dissection.

Sample

After the Home position has been set, pressing the black *Sample* button (**Figure 2**) will initiate sample collection by bringing the DCU down to the Home position and activating the vacuum at the selected strength and duration.

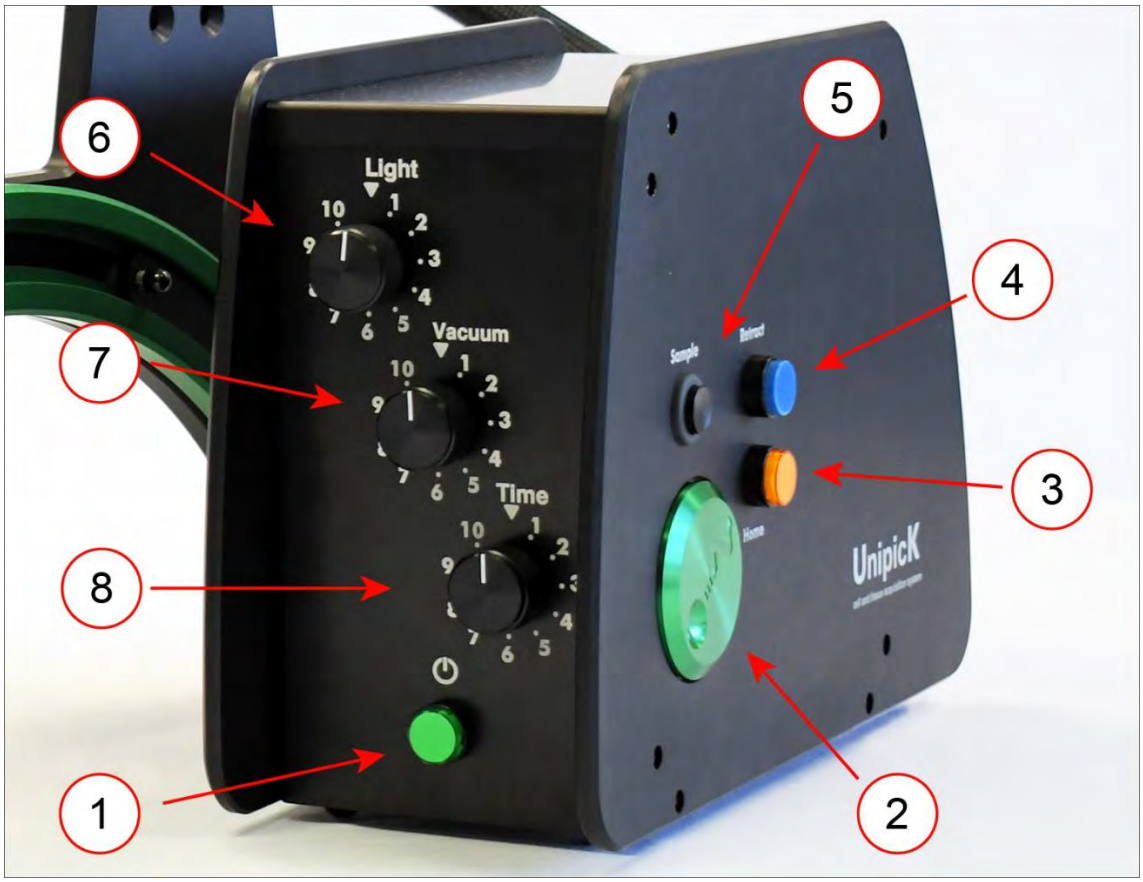


Figure 2: ***UnipicK™*** *control box includes: (1) Power on/off (green button); (2) DCU Positioning (green knob): to bring the DCU up or down towards the microscope stage; (3) Home (orange button): to calibrate capillary (DCU) for cell and tissue collection and set it in standby position; (4) Retract (blue button) to automatically move the capillary above the plate to allow collection from different wells without recalibration procedure. Subsequent pressing of the Retract button brings the DCU back to a previously calibrated Standby position; (5) Sample (black button): for cell/tissue collection; (6) Light intensity (top dial); (7) Vacuum strength (middle dial): increasing vacuum pressure from 1 (minimum, 2 inches of mercury) to 10 (maximum, 22 inches of mercury); (8) Impulse Duration (bottom dial): increasing vacuum duration in increments of 100 milliseconds, from 1 (shortest, 100 milliseconds) to 10 (longest, 1 second).*

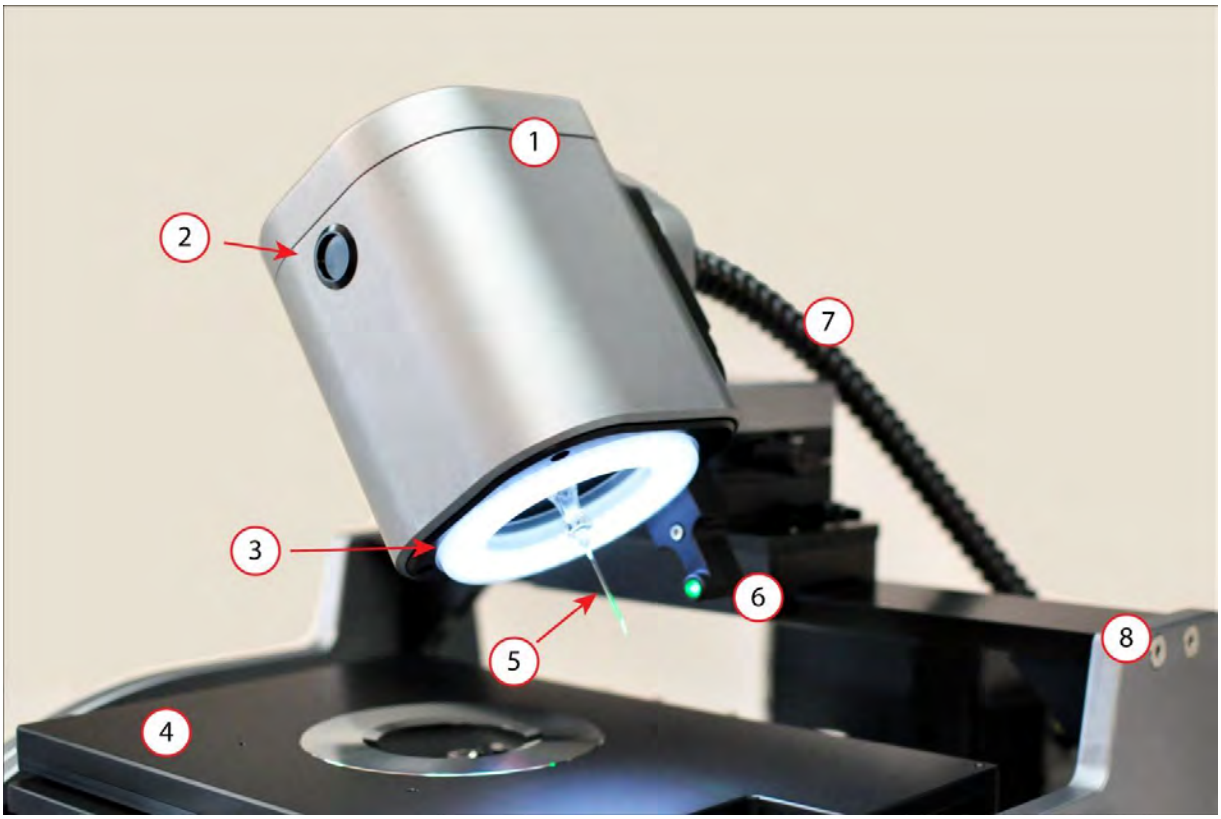


Figure 3: ***UnipicK™*** *sample collection Head (1) in its half-turned position. The following components are shown: (2) On/off switch button for green horizontal calibration light; (3) adjustable ring light for sample illumination; (4) microscope x-y stage; (5) attached DCU with projected green calibration light; (6) green horizontal calibration light used for DCU tip identification during calibration procedure; (7) Vacuum Line and Electrical Cables; (8) Universal Straddle. The Head should be turned full 90˚ for disposable capillary (DCU) attachment/removal. The x-y position of the DCU is controlled by the knobs on the linear stages/micromanipulators (not shown). The knob on the bottom stage control the side to side movement and the knob on the top stage control the front to back movement of the DCU for the purpose of centering it above the mechanical x-y stage.*

Initial Training Exercises with UnipicK™

For instructional videos visit our website: www.ndx-instruments.com or YouTube channel: http://www.youtube.com/NDXInc

1. Start **UnipicK™** by pressing the green *Power* button once (green button will light up when depressed).

Caution: Wear gloves and protective eyewear when handling DCUs containing glass capillaries.

2. Place a marking on a histological glass slide and place it on the mechanical stage with 2 drops of distilled water.
3. To attach the filter and DCU; rotate the Sampler Head 90˚clockwise, and attach a filter directly to the male luer connecter on the **UnipicK™** Sampler Head. Carefully handle new DCU by its hub base and attach it directly onto the filter.

***NOTE:* Green light on the Head is used to visualize the green annulus of the DCU under the microscope. When attaching a filter and DCU, green light may be turned off. It must be turned on again before lowering the DCU for calibration.**

4. Rotate **UnipicK™** Sampler Head clockwise so that the DCU tip points down.
5. Using the green knob, carefully bring the DCU down until the capillary tip touches the surface of the liquid. **Optional:** Press the blue *Retract* button to bring the DCU tip down by 15 mm first before using the green knob.

***NOTE:* Slowly turning the green knob will move the capillary 1.5 µm per step indicated by a clicking sound. Turn the green knob fast to move the capillary at 225µm/s.**

6. When in focus, the annulus of the capillary tip appears as a bright green ring.
7. Use the linear stages/micromanipulators (**Figure 3**) to center the green annulus ring. It is recommended to locate the green annulus under the lowest magnification first.
8. Locate the marking on slide. Using coarse and fine focus adjustment knobs to focus on marking.
9. Carefully bring the DCU down until the annulus of the capillary tip comes into focus as a bright green ring.

10. Stop lowering the DCU when the capillary tip moves with the glass slide when using the mechanical stage. It is helpful to move the slide/plate using the mechanical stage to determine if the DCU tip has made contact with the slide/plate surface. When the DCU tip is in contact with the surface, a slight motion of the mechanical stage will result in a movement of the capillary tip.
11. Press the blue *Retract* button to bring the DCU to its highest position and repeat the process from step 6.

DCU Calibration for Tissue Microdissection

Each new DCU must be calibrated before sampling. For instructional video visit our website: www.ndx-instruments.com or our YouTube channel: http://www.youtube.com/NDXInc

NOTE: **Depending on the region of interest, DCUs of different internal diameters (IDs) should be used. The size of desired cells and cell clusters shall determine the appropriate diameters of the DCUs. An average mouse interneuron is about 15-25µm. For microdissection of brain anatomical regions (e.g. mouse hippocampus) ~50µm ID DCU is recommended. Subanatomical regions, such as dentate gyrus or cortical layers, may be collected with ~30µm ID DCUs. If fine microdissection is required, DCUs with <30µm diameters should be used.**

Wear gloves and protective eyewear when handling DCUs containing glass capillaries.

1. Start **UnipicK™** by pressing the green *Power* button once (green button will light up when depressed)
2. Place a tissue slide on the microscope stage and add buffer to the tissue.
3. Position the slide to a clear spot on the slide adjacent to the tissue sample for calibration.
4. Rotate the **UnipicK™** Sampler Head 90˚ counterclockwise (**Figure 3**) to attach a filter directly to the male luer connecter on the **UnipicK™** Sampler Head. Then carefully handle a new DCU by its hub base and attach it directly onto the filter. [Green light on the Head is used to visualize the green annulus of the DCU under the microscope. When attaching a filter and DCU, green light may be turned off. It must be turned on again before lowering the DCU for calibration.]

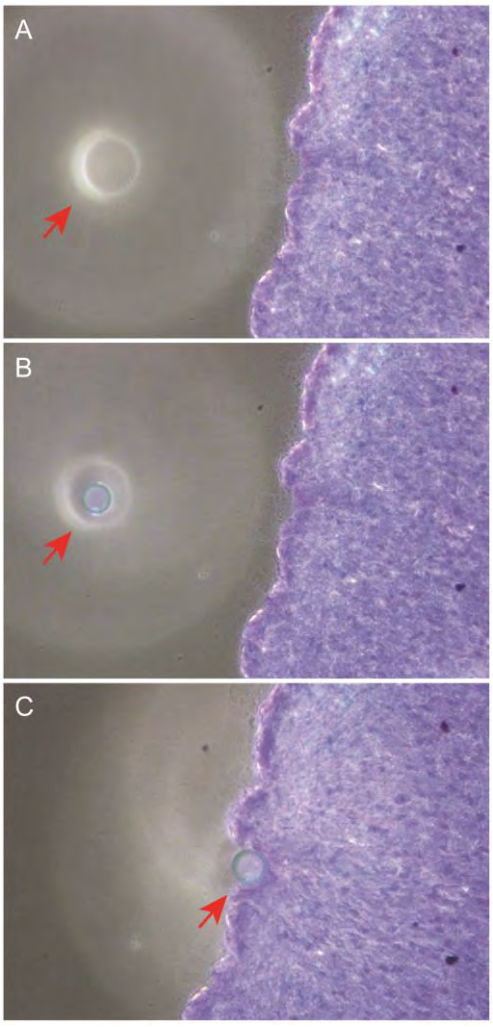
Caution: DCUs have sharp tips! Handle with care. Improper handling may cause injuries. Never contact the capillary tip to avoid injury and contamination.

Caution: Always use a filter with the DCU to prevent biological material and liquids from entering UnipicK's mechanical parts. When UnipicK™ is not in use, attach a new filter unit to prevent foreign objects from entering the system until next use.

5. Rotate **UnipicK™** Head clockwise so that the DCU tip points down.
6. Using the green knob, carefully bring the DCU down until the capillary tip touches the surface of the buffer.

***NOTE:* Slowly turning the green knob will move the capillary 1.5 µm per step indicated by a clicking sound. Turn the green knob fast to move the capillary at 225µm/s.**

7. Use the linear stages/micromanipulators to locate the DCU halo and position the halo adjacent to the tissue (**Figure 4A**). It is recommended to locate the green annulus under the lowest magnification.



8. Use coarse and fine focusing knobs to focus on the tissue. Slowly bring the DCU down until the annulus of the capillary tip comes into focus as a bright green ring (**Figure 4B**). Lower the DCU until the capillary tip comes in contact with the tissue (**Figure 4C**). It is helpful to move the microscope slide with the mechanical stage in order to determine if the DCU tip has made contact. When the DCU tip is in contact with the slide surface, a slight motion of the mechanical stage will result in the simultaneous movement of the capillary tip. If the tug of the slide movement is observed, lift the DCU up by turning the green knob slowly for a few clicks until the motion of the mechanical stage no longer pulls the capillary tip. This assures that the DCU's lowest position will not break the tip upon downward movement during dissection
9. Using the linear stages/micromanipulators align the annulus of the capillary tip to the center of the ocular crosshairs**.**

Figure 4: *Three step procedure for DCU calibration.* ***A:*** *Initial finding of DCU tip (halo, shown with red arrow) in the field of vision;* ***B:*** *Lowering the DCU until the DCU tip and tissue section are both in focus;* ***C:*** *Moving the x-y mechanical stage to test if the DCU tip is in contact with the tissue section surface.*

Caution: Bringing down the DCU too low will cause the capillary tip to break against the sample slide or plate. If you suspect that a tip has been broken, be sure to check the surrounding area for broken glass before replacing the DCU to avoid injury or damage.

10. Press the orange *Home* button (orange button will light up when calibrated) to set the calibrated Home position. The DCU will lift to the Standby position 1 mm above the sampling position.
11. To re-calibrate, simply press the green knob to bring the DCU back to Home position. Re-position the capillary tip using the green knob and then push the orange button to set the new Home position. To cancel re-calibration, press the green knob instead of pressing the Home button. This will bring the DCU back up to the previous Standby position.
12. You may re-calibrate at any time during the sampling procedure to designate a new Home position.
13. Proceed to **Cell/Tissue Collection Section**

Caution: When the UnipicK™ Head is rotated 90˚ counterclockwise, the DCU will not return to its initial Starting position. To return the DCU to the initial Starting position, press the blue *Retract* button. To avoid damaging the DCU tip we recommend returning the DCU to its initial Starting position before removing it from the unit.

DCU Calibration for Adherent Cell Cultures

NOTE: **Each new DCU must be calibrated before sampling. For instructional video visit our website: www.ndx-instruments.com or YouTube channel: http://www.youtube.com/user/NDXInc**

Caution: Wear gloves and protective eyewear when handling DCUs containing glass capillaries.

TIP: When working with attached cells it is recommended to wash cells with fresh medium to remove floating cells.

1. Start **UnipicK™** by pressing the green *Power* button once (green button will light up when depressed)
2. Place a cell plate/dish on the microscope stage.

3. Rotate the **UnipicK™** Head 90˚ counterclockwise (**Figure 3**) to attach a filter and DCU. Attach a filter directly to the male luer connecter on the **UnipicK™** Sampler Head. Then carefully handle a new DCU by its hub base and attach it directly onto the filter.

Caution: DCUs have sharp tips! Handle with care. Improper handling may cause injuries. Never contact the capillary tip to avoid injury and contamination.

Caution: Always use a filter with the DCU. The filter will prevent biological material and liquids from entering UnipicK's mechanical parts. When UnipicK™ is not in use, attach a new filter unit to prevent foreign objects from entering until next use.

4. Rotate **UnipicK™** Head clockwise so that the DCU tip points down. By turning the green knob, carefully bring the DCU down until the tip of capillary touches the surface of the liquid.

NOTE: **Slowly turning the green knob will move the capillary 1.5 µm per step indicated by a clicking sound. Turn the green knob fast to move the capillary at 225µm/s. Use caution.**

5. Use the linear stages/micromanipulators (**Figure 3**) to locate the DCU halo.
6. Use coarse and fine focusing knobs to focus on the cells. Slowly bring the DCU down until the annulus of the capillary tip comes into focus as a green ring. Position the green ring over a clear spot. Continue to lower the DCU until the tip has made contact with a cell. It is helpful to move the plate/dish with the mechanical stage in order to determine if the DCU tip has made contact with a cell or the plate/dish surface. If the DCU tip is just above the slide surface, the edge of the cell will press against the capillary tip (Similarly to **Figure 4**).
7. When the DCU tip is in contact with the culture plate surface, a slight motion of the mechanical stage will result in the simultaneous movement of the capillary tip. If the tug of the culture plate movement is observed, lift the DCU up by turning the green knob slowly for a few clicks until the motion of the mechanical stage no longer pulls the capillary tip. This assures that the lowest position of the capillary tip will not break the tip upon downward movement during dissection.
8. Using the linear stages/micromanipulators align the annulus to the center of the ocular crosshairs (**Figure 3**).

Caution: Bringing down the DCU too low will cause the capillary tip to break against the sample slide or plate. If you suspect that the tip has been broken, be sure to check the surrounding area for broken glass before replacing the DCU to avoid injury or damage.

9. Press the orange *Home* button (orange button will light up when calibrated) to set the calibrated Home position. The DCU will lift to the Standby position 1 mm above the sampling position.
10. To re-calibrate, simply press the green knob to bring the DCU back to Home position. Re-position the capillary tip using the green knob and then push the orange button to set the new Home position. To cancel re-calibration, press the green knob instead of pressing the *Home* button. This will bring the DCU back up to the previous Standby position.
11. You may re-calibrate at any time during the sampling procedure to designate a new Home position.
12. Proceed to **Cell/Tissue Collection Section**

Caution: When the UnipicK™ Head is rotated 90˚ counterclockwise, the DCU will not return to its initial Starting position. To return the DCU to the initial position, press the blue *Retract* button. To avoid breaking DCU tip we recommend returning DCU to its initial Starting position before removing it from the unit.

Cell/Tissue Collection

1. Adjust values for the white LED ring light, vacuum pressure, and duration.

***NOTE:* Optimal vacuum levels and duration can vary depending on the type of cell culture, cell confluence, and tissue type. It is recommended that a test sample be used to determine the optimal values of vacuum level and duration prior to sampling. Start at the lowest setting for vacuum level and duration and slowly increase them in turn.**

2. Position the cell plate/tissue slide to an area for acquisition. Under the microscope, the annulus of the capillary tip is where the cell/tissue will be collected.
3. Press the black *Sample* button to acquire the targeted cell/tissue.
4. Working quickly, repeat until the desired number of cells/amount of tissue has been collected or until DCU has reached its capacity. **Stop when the capillary shaft is filled just below the hub with buffer.**
5. Make sure that a syringe with a male luer lock has been prepared with the plunger slightly pulled back and a filter attached to the tip (**Figure 5**). To empty the contents from the DCU, rotate the **UnipicK™** Head 90˚ counterclockwise and carefully detach the DCU. Affix the DCU to the filter on the prepared syringe (**Figure 5**). Using both hands

hold the DCU in a receptacle (e.g. microcentrifuge tube) and eject contents by gently pushing the plunger down.

6. Attach a DCU to **UnipicK™** Head and re-calibrate to continue collection.

Retract Function

The Retract feature remembers the most recent Home position, allowing the user to dispense contents of the DCU or move between wells without having to recalibrate.

To Move Between Wells Using the Same DCU:

1. Press the blue *Retract* button. This will bring the DCU up to its original Starting position.
2. Move the plate using the mechanical stage to position the next well under the field of view.
3. Press the blue *Retract* button again to return the DCU to its Standby position.
4. Press the green knob to bring the DCU down from the Standby position to the most recently set Home position. If necessary, align the annulus of the capillary tip to the center of the ocular crosshairs using the linear stages/micromanipulators and/or adjust the z-axis position of the DCU using the green knob.

NOTE: In some instances, the DCU will have to be raised or lowered slightly using the green knob due to slight discrepancies in plate height.

5. Press the orange *Home* button to designate the new Home position. The DCU will move up to the new Standby position.
6. Resume collection.

Alternative Procedure:

1. From the Standby position, press the green knob to bring the DCU down to the most recently set Home position. This will cancel the Home position.
2. Using the green knob, move the DCU slightly up. (We recommend this step to avoid DCU breakage.)
3. Press the orange *Home* button to set the new Home position.
4. Press the blue *Retract* button to move the DCU up to the Starting position.
5. Move the plate using the mechanical stage to position the next well under the field of view.

6. Press the blue *Retract* button again to return the DCU to its Standby position.
7. Press the green knob to bring the DCU down from the Standby position to the most recently set Home position. If necessary, align the annulus of the capillary tip to the center of the ocular crosshairs using the linear stages/ micromanipulators, and adjust the z-axis position of the DCU by turning the green knob to the desired Home position.
8. Press the orange *Home* button to designate the new Home position and bring the DCU up to the Standby position.
9. Resume collection.

To Dispense DCU Contents and Reuse the Same DCU:

1. Press the blue *Retract* button. This will bring DCU up to its original Starting position.
2. Rotate the **UnipicK™** Head 90˚ counterclockwise to access the DCU.
3. Press the *Retract* button again to bring the DCU out for easier access.
4. Remove the DCU and dispense contents using a syringe as previously described (**Figure 5**).
5. Attach the DCU to the filter on the Head and press the *Retract* button. This will raise the DCU to its Starting position.
6. Rotate the **UnipicK™** Head clockwise so that the DCU tip points down.
7. Press the green knob to bring the DCU down to the most recently set Standby position. This will cancel the previously set Home position (the orange *Home* button light will turn off).

NOTE: **In some instances, the DCU will have to be raised or lowered slightly using the green knob due to slight discrepancies in plate height.**

8. Align the annulus of the capillary tip to the center of the ocular crosshairs using the linear stages/micromanipulators and/or adjust the z-axis position of the DCU by turning the green knob to determine the new Home position.
9. Press the orange *Home* button to designate the new Home position. The DCU will move up to the new Standby position.
10. Resume collection.

Alternative Procedure:

1. From the Standby position, press the green knob to bring the DCU down to the most recently set Home position. This will cancel the Home position.
2. Using the green knob, move the DCU slightly up. (We recommend this step to avoid DCU breakage.)
3. Press the orange *Home* button to set the new Home position.
4. Press the blue *Retract* button to move the DCU up to the Starting position.
5. Rotate the **UnipicK™** Head 90˚ counterclockwise to access the DCU.
6. Press the *Retract* button again to bring the DCU out for easier access.
7. Remove the DCU and dispense contents using a syringe as previously described (**Figure 5**).
8. Attach the DCU to the filter on the Head and press the *Retract* button. This will raise the DCU to its Starting position.
9. Rotate the **UnipicK™** Head clockwise so that the DCU tip points down.
10. Press the green knob to bring the DCU down to the most recently set Home position. This will also cancel the previously set Home position (the orange *Home* button light will turn off).
11. Align the annulus of the capillary tip to the center of the ocular crosshairs using the linear stages/micromanipulators, and adjust the z-axis position of the DCU using the green knob to determine the new Home position.
12. Press the orange *Home* button to designate the new Home position. The DCU will move up to the new Standby position.
13. Resume collection.

Changing DCUs

NOTE: **Because of the slight variability in the lengths of the DCUs avoid the use of this feature when switching from one DCU to another to prevent accidental breakage of the DCU.**

1. Press the blue *Retract* button to lift the DCU up to its original Starting position.
2. Rotate the **UnipicK™** Head 90˚ counterclockwise to access the DCU.
3. Press the *Retract* button again to bring the DCU out for easier access.
4. Remove the DCU. Dispense the contents using a syringe (**Figure 5**) if desired.
5. Attach the new DCU to the filter on the Head and press the *Retract* button. This will raise the DCU to its Starting position.

6. Rotate the **UnipicK™** Head clockwise so that the DCU tip points down.
7. From the starting position, press the green knob to bring the DCU down to the most recently set Standby position. This will cancel the previously set Home position (the orange *Home* button light will turn off).
8. Align the annulus of the capillary tip to the center of the ocular crosshairs using the linear stages/micromanipulators and/or adjust the z-axis position of the DCU turning the green knob to determine the new Home position.
9. Press the orange *Home* button to designate the new Home position. The DCU will move up to the new Standby position.
10. Resume collection.

Alternative Procedure:

1. From the Standby position, press both *Positioning* buttons simultaneously to bring the DCU down to the most recently set Home position. This will cancel the Home position.
2. Press the blue *Retract* button to lift the DCU up to its original Starting position.
3. Rotate the **UnipicK™** Head 90˚ counterclockwise to access the DCU.
4. Press the *Retract* button again to bring the DCU out for easier access.
5. Remove the DCU. Dispense the contents using a syringe (**Figure 5**) if desired.
6. Attach the new DCU to the filter on the Head and press the *Retract* button. This will raise the DCU to its Starting position.
7. Press the blue *Retract* button again to move the DCU down closer to the slide/plate.
8. Once the DCU has stopped moving, continue to bring the DCU down to a new Home position using the green knob.
9. Press the orange *Home* button to designate the new Home position. The DCU will move up to the new Standby position.
10. Resume collection.

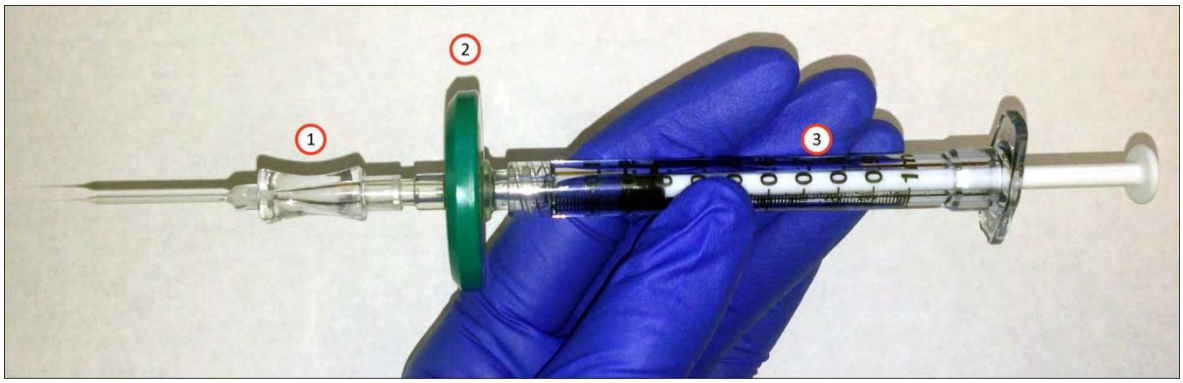


Figure 5: *DCU and filter affixed to a luer lock sterile syringe. 1 – DCU; 2 – filter, 3 – syringe.*

6 Sample Protocols

Protocol 1: Collection of Individual Adherent Cells from Culture Dishes

Various adherent cell cultures including human neuroblastoma cell line SH-SY5Y, Chinese hamster ovary (CHO) cells, human melanoma MDA-MB-435 cells and various primary cultures, such as neural progenitors, skin fibroblasts, etc., can be used for the collection of individual cells using **UnipicK™**. The collected cells are viable and may be used for recultivation and clonal expansion, as well as for single cell analysis, including protein and nucleic acid analyses. See Application Notes on our website: http://www.ndx-instruments.com

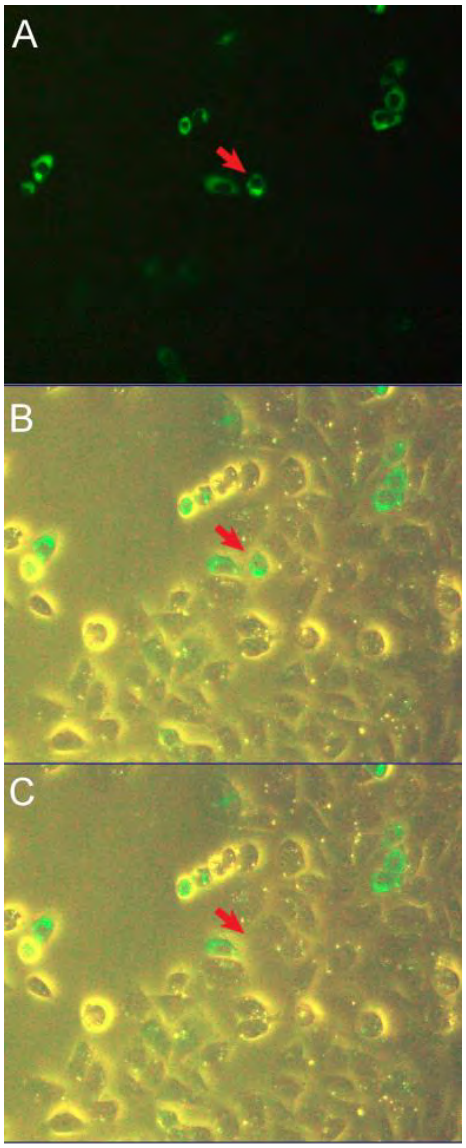
NOTE: **Recommended medium for neuroblastoma cell line SH-SY5Y, Chinese hamster ovary (CHO) cells and human melanoma MDA-MB-435 cells (ATCC, Manassas, VA): Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) (Life Technologies, Grand Island, NY) supplemented with 10% (v/v) heat-inactivated fetal calf serum containing 2mM glutamine and 1% antibiotics (penicillin–streptomycin). Cells should be maintained at 37°C in a humidified 5% CO_2 atmosphere.**

Cell collection with UnipicK™ from cultures: It is recommended for cell cultures to not exceed 50% confluence for single cell collection, although collections may be performed at higher confluence. Prior to cell collection, media should be removed from the plate/dish and cells should be gently washed once with fresh pre-warmed medium to remove dead floating cells. Replenish media and place plate/dish on microscope stage. Proceed with cell collection as described in **Calibration for Cell Cultures** and **Cell Collection** sections.

Selecting DCU Size: DCU size should be selected based on the size of cells of interest and the confluence of cultures. DCUs with internal diameter (ID) greater than the cells are recommended when collecting cells for recultivation and clonal expansion, i.e. when collecting cells of 20µm diameter, a DCU with ID 30µm should be used to maximally maintain cell viability. DCUs with <30µm diameters is recommended when working with smaller cells or cultures with >70% confluence. However, DCUs with smaller ID may cause some damage to cells.

Collecting Individual Cells: To collect cells, position the desired cell under the center of crosshairs and press the black *Sample* button. DCU is lowered and cell is aspirated into the capillary. It is recommended that no more than 30 minutes be spent per plate. However, timeframe for cell collection is dependent on cell type and sensitivity.

When collecting fluorescently labeled cells (**Figure 6**), calibrate the DCU under bright field as described above, and then turn off LED ring light. Turn on the fluorescent illumination to locate desired labeled cells. Turn the LED ring light on and position the cell under the center of the crosshair. Turn fluorescent illumination off to prevent bleaching of cells. After collecting the cell, fluorescent illumination may be turned on again and the process repeated.



NOTE: **Optimal vacuum levels and duration can vary depending on the type of cell cultures and confluence of the cells. It is recommended that a test cell culture dish be used to determine the optimal values of vacuum level and duration prior to sampling. Start at the lowest settings for vacuum level and duration and slowly increase them in turn.**

Ejecting Cells From DCU: Prior to removing a DCU from **UnipicK™** Head, pull the syringe plunger back and attach a filter to the syringe tip (**Figure 5**). Press the blue *Retract* button to bring the DCU up to the Starting position. Carefully remove the DCU from **UnipicK™** Head and affix to the filter on syringe. Carefully position the DCU tip into preloaded media or buffer and slowly eject cells.

Figure 6: *Collecting fluorescently labeled cells.* ***A:*** *Initial identification of fluorescently labeled cells under fluorescent illumination.* ***B:*** *Under bright field fluorescent label is still visible. Bright field is used to position the cell of interest under the crosshair for collection and to visualize cell collection into the DCU. Fluorescent illumination should be turned off to prevent bleaching of label prior to sampling.* ***C:*** *Upon collecting the cell, turn on fluorescent illumination to resume collection.*

NOTE: **Change the DCU and filter after each collection procedure. DCUs may be reused by washing with a syringe and sterilizing with ethanol. DCUs can also be autoclaved. However, NeuroInDx recommends using a new DCU for each collection procedure to avoid contamination.**

Protocol 2: Sucrose Treated Frozen Brain Tissue Microdissection

This protocol describes the isolation of single cells, cell clusters, and subanatomical regions from sucrose treated brain slices (**Figure 7**). Sucrose treated brain tissue keeps brain morphology intact, and thus ideal for microdissection. Furthermore, this optimized protocol yields high quality RNA from the microdissected material.

Materials:

- Surgical instruments
- Standard animal perfusion apparatus and setup
- Cryostat
- Standard Phosphate Buffered Saline (PBS)
- Sucrose (15-20%) in PBS filtered
- 2-methylbutane
- Dry ice
- Glass microscope slides
- Cresyl violet, toluidine blue, hematoxylin, or any vital dyes
- Pipettor and sterile pipette tips
- **UnipicK™** disposable capillary units (DCU) with appropriate internal diameter (ID) for the application. ID≤30 µm single cell collection. ID≈20-100 µm for subanatomical regions.

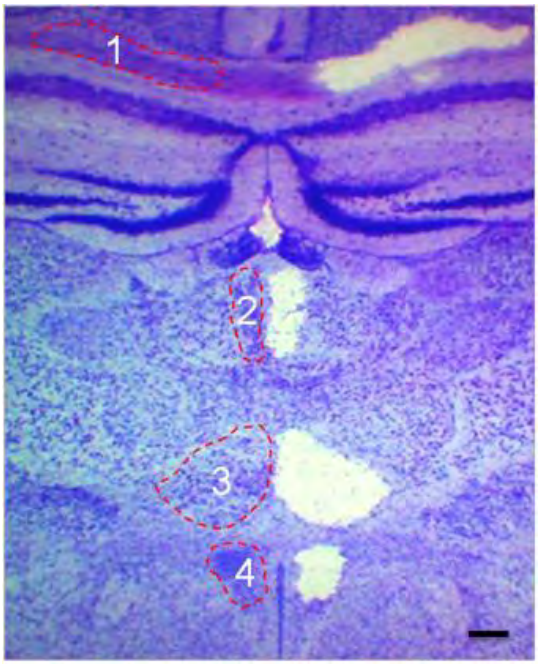
Recommended tissue preparation:

Flush animal with standard pre-perfusion PBS (phosphate buffer saline). Remove the brain and sink in 15-20% Sucrose in PBS at 4°C overnight. Flash freeze the brain in 2-methylbutane on dry ice. If not for immediate use, store tissue at -80°C. Prepare cryosections from 10 to 100 µm thickness. For single cell collection with **UnipicK™**, cut sections at ≤30 µm. Stain slides with 0.01% toluidine blue for 10 seconds. Most standard dyes may be used for staining brain sections at lower than standard concentrations. Avoid over staining, as this will cause the sample to become stiff, thus difficult to microdissect. Wash with standard PBS. Also, see Application Notes on our website: http://www.ndx-instruments.com/support/downloads.

Microdissection or Single Cell Collection:

Dry the back of the slide after PBS wash and place the slide on **UnipicK™** microscope stage. It is critical to keep the tissue section moist at all times by adding either PBS or 15% sucrose in PBS during longer sessions of microdissection. High quality RNA may be isolated from tissue samples collected within 1 hour. Attach a DCU to **UnipicK™** Head. Follow instructions in **DCU Calibration for Tissue Microdissection** and **Cell/Tissue Collection** sections.

NOTE: **Optimal vacuum levels and duration can vary depending on the type of samples being dissected. It is recommended that a test slide be used to determine the optimal values of vacuum level and duration prior to sampling. Start at the lowest settings for vacuum level and duration and slowly increase them in turn.**



Selecting DCU Size: DCUs of different diameters should be used based on the size of the cells and subanatomical regions of interest. ID≤30 µm for single cell collection and ID≈20-100µm for subanatomical regions is recommended.

Figure 7. *Unilateral microdissection of the corpus callosum (1), paratenial thalamic nucleus (2), nucleus reunions (3) and hypothalamic nucleus (4). Tissue was stained with toluidine blue. Tissue thickness 50 µm. Scale bar= 250µm*

NOTE: **Check the DCU carefully during dissection to make sure that the samples are being collected into the capillary. If not, repeat calibration or adjust vacuum level and duration.**

Caution: Stop when the capillary shaft is filled to <u>just below the hub</u> with buffer. Detach DCU and transfer collected samples as described above to avoid liquid transfer beyond the filter into the system.

Ejecting Cell/Tissue from DCU:

Prior to removing a DCU from **UnipicK™** Head, pull the syringe plunger back and attach a filter to the syringe tip. Carefully remove the DCU from **UnipicK™** Head and affix to the filter on syringe. Carefully position the DCU tip into preloaded buffer and slowly eject cell/tissue. Cells/tissue may be ejected into a microcentrifuge tube containing the desired buffer for subsequent application. To remove remaining tissue inside the capillary shaft, carefully and slowly load the attached syringe with PBS to rinse the DCU and then release the contents into the microcentrifuge tube. If not for immediate use, samples may be frozen on dry ice and then stored at -80°C.

NOTE: **Change the DCU and filter after each collection procedure. DCUs may be reused by washing with a syringe and sterilizing with ethanol. DCUs can also be autoclaved. However, NeuroInDx recommends using a new DCU for each collection procedure to avoid contamination.**

Protocol 3: Microdissection of Native Brain Tissues

UnipicK™ is able to dissect live tissues for primary culture and other various downstream molecular analyses. The following is a sample protocol for the collection of live cells from brain tissue to be used for culturing. **UnipicK™** can be applied to dissect live cells from other tissues, such as tumors, for primary culture using suitable protocols. Under appropriate extracellular and sterile conditions ensuring cell viability, fresh adult brain tissue containing live cells may be used for dissection for up to 8 hour.[1] See Application Notes on our website: http://www.ndx-instruments.com/support/downloads.

Materials:

- Vibratome
- Thermostat bath
- Surgical instruments
- Artificial Cerebrospinal Fluid (ACSF) 300-500ml containing (in mM): 126 NaCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, and 10 D-glucose, pH 7.4, carbogen (95% O_2 5% CO_2). ACSF may be replaced by tissue culture medium balanced to carbogen.
- Dissociation solution: 1mM EDTA, 0.05% Trypsin
- Hanks Balanced Salt Solution (HBSS)
- Neurobasal media with supplements and growth factors: Neurobasal media (NB+++, see below) containing 1% (v/v) Penicillin/Streptomycin; 2µM (final concentration) L-glutamine; 0.04% (v/v) Heparin, 2% (v/v) B-27; 20ng/ml fibroblast growth factor, FGF2; and 10ng/ml epidermal growth factor, EGF.
- Ice

***NOTE:* ACSF may be prepared the night before and cooled to 4°C in a refrigerator.**

Tissue Preparation: Specimen preparation may be performed according to standard protocols. Described below is the preparation used for **UnipicK™** protocol.

1. Aerate ACSF with carbogen 10-20 minute before the experiment.
2. Anesthetize the animal with 50 mg/kg Nembutal or other anesthetic approved by your ARC.
3. Open the chest and flush the animal through the aorta with cold aerated ACSF for 3-5 minutes.
4. Excise the brain, rinse with cold ACSF, and remove excess liquid with sterile napkin.

[1] Practical Electrophysiological Methods: A Guide for In Vitro Studies in Vertebrate Neurobiology. (1992) Eds. Helmut Kettenmann and Rosemarie Grantyn, Wiley-Liss, New York

5. Keep brain in buffer consisting of Krebs-Ringer solution saturated with carbogen (95% O_2, 5% CO_2) on ice until mounted onto glass slides.
6. Glue the brain on the vibratome platform with fast glue (e.g. medical device adhesive Loctide 4014).
7. Slice 200-300µm sections and carefully place a tissue slice onto a glass slide and keep moist with ACSF. Keep slides on ice.

Tissue Microdissection and Collection:

8. Dry the back of the slide and place it on UnipicK™ microscope stage. Cover the slide surface with aerated ACSF using a dropper or a pipettor and follow instructions in **DCU Calibration for Tissue Microdissection** and **Cell/Tissue Collection** sections.

Caution: Stop when the capillary shaft is filled to just below the hub with buffer. Detach DCU and transfer collected samples as described above to avoid liquid transfer beyond the filter into the system.

NOTE: **DCUs with ID 100µm diameter and greater are recommended for adult rat brains.**

NOTE: **For sample dissection video visit our website: www.ndx-instruments.com or YouTube channel: http://www.youtube.com/user/NDXInc**

Ejecting Cell/Tissue from DCU:

9. Prior to removing a DCU from **UnipicK™** Head, pull the syringe plunger back and attach a filter to the syringe tip.
10. Carefully remove the DCU from **UnipicK™** Head and affix to the filter on syringe.
11. Carefully position the DCU tip into preloaded buffer and slowly eject tissue into a 1.5mL tube.
12. Add 50 µL of 0.05% trypsin.
13. Incubate at 37°C for 10 minutes.
14. Add 450 µl of Hank's balanced saline solution (HBSS) and centrifuge at 1500 rpm for 5 minutes.
15. Remove the supernatant.
16. Add 800 µl of NB+++ and dissociate cells by aspirating with a pipette.
17. Plate the cells and incubate at 37°C in a 5% CO_2 atmosphere.

Media

NB+++ (50 ml)

Neurobasal medium	48 ml
Penicillin/Streptomycin (100X)	500 µl
L-glutamine (200 mM), [2 mM final]	500 µl
Heparin (5 mg/ml), [2 µg/mL final]	20 µl
B-27 (50X)	1 ml
FGF2 (25 ng/µl), [20 ng/ml final]	40 µl
EGF (100 ng/µl), [10 ng/ml final]	5 µl

Protocol 4: Microdissection of Microcapillary Cell Walls from Mouse Heart Muscle Tissue

This protocol is suitable for dissecting most difficult–to-dissect tissue and isolating high quality RNA from collected samples.

Materials:

- Liberase DL Research Grade (Roche, REF 05-401-160-001), 5mg vial
- Proteinase inhibitor (Sigma tablets; SigmaFast S8820)
- Kolliphor® P188 (Sigma 15759) also known as Pluronic F-68
- 1M sterile $CaCl_2$
- MEM without L-glutamine, FBS, or antibiotics (Corning)
- 1x Phosphate buffer saline (PBS)
- Cryostat
- Ice

Reagents:

Prepare prior to the day of microdissection:

1. **P188 stock solution** (15mM; 100x stock solution), place in a 37˚C water bath to dissolve, filter sterilize and store at 4˚C in the dark
2. **Liberase DL Stock Solution**
    a. Slowly thaw enzyme (Liberase DL, lyophilized form) on ice for 30 min
    b. Add 2ml of MEM, alternatively 1x PBS may also be used
    c. Keep on ice, resuspend by pipetting. Do not vortex. Stock may be kept at 4°C for up to a month. Alternatively, aliquot into smaller volumes (200 µl) and store at -20°C. Use or discard an aliquot after thawing. Do not re-freeze

<u>Prepare on the day of dissection</u>

1. **Dissociation Solution** by mixing the following components to make 10ml of solution
    a. 200µl Liberaze stock solution
    b. 75µl 1M $CaCl_2$
    c. 100µl P188
    d. MEM may be replaced by 1xPBS to 10ml
    e. Place on ice
2. **Inhibitor Solution**
    a. 1 proteinase inhibitor tablet in 250ml MEM or PBS

Tissue Preparation:

1. Anesthetize the animal with 50 mg/kg Nembutal or other anesthetic approved by your ARC.
2. Flush/perfuse the animal with standard PBS. DO NOT USE ANY FIXATIVES.
3. Remove the heart and other organs and sink them in 15-20% Sucrose in PBS at 4°C overnight.
4. Flash freeze heart and organs in 2-methylbutane on dry ice. If not for immediate use, store tissue at -80°C.
5. Prepare cryosections from 10 to 25µm thickness.
6. Stain slides with 0.025% cresyl violet for 10 seconds and gently wash with PBS.
7. Apply Dissociation Solution to sections and incubate at room temperature for no more than 30 minutes.
8. Tilt the slide onto an absorbent surface (e.g. paper towel) and soak away the dissociation solution.
9. Gently wash tissue sections with Inhibitor Solution.

Tissue Microdissection and Collection:

10. Dry the back of the slide and place it on **UnipicK™** microscope stage.
11. Cover the slide surface with Inhibitor Solution using a dropper or a pipettor and follow instructions in **DCU Calibration for Tissue Microdissection** and **Cell/Tissue Collection** sections.

Caution: Stop when the capillary shaft is filled to just below the hub with buffer. Detach DCU and transfer collected samples as described above to avoid liquid transfer beyond the filter into the system.

NOTE: **Selecting DCU Size: DCUs with ID≤30µm for single cell collection, ID≈20-100µm for subanatomical regions, and ID≈60-80µm for microcapillaries are recommended.**

NOTE: **For sample dissection video visit our website: www.ndx-instruments.com or YouTube channel: http://www.youtube.com/user/NDXInc**

Ejecting Cell/Tissue from DCU:

12. Prior to removing a DCU from **UnipicK™** Head, pull the syringe plunger back and attach a filter to the syringe tip.
13. Carefully remove the DCU from **UnipicK™** Head and affix to the filter on syringe.
14. Carefully position the DCU tip into a microcentrifuge tube preloaded with buffer, and slowly eject cell/tissue. If there is remaining tissue inside the capillary shaft, carefully and slowly load the attached syringe with Inhibitor Solution to rinse the DCU and then release the contents into the microcentrifuge tube.
15. Spin cells at 500 x g for 5 minutes and remove the supernatant to remove the Inhibitor Solution.
16. Samples may then be used immediately or frozen on dry ice and stored at -80°C for later use.

7 Troubleshooting

If problems with **UnipicK™** occur by factors other than manufacturing defects, please review the following guide. If you encounter any other problems, please contact technical support.

Problem	Cause	Solution
1. Tissue or cell is not being picked up into the DCU.	Vacuum level is not high enough.	Increase vacuum pressure and/or duration.
		Use a DCU with larger tip diameter.
	DCU tip is clogged.	Maintain humidity between tissue and the DCU tip.
		Change to a new DCU.
	DCU tip is not touching the sample slide.	Re-calibrate the DCU.
	DCU tip has been damaged.	Change to a new DCU.
2. Too much background light for capturing microscope images.	Green horizontal LED light on **UnipicK™** Head.	Press the black button on the **UnipicK™** Head to turn the green LED off. Button should be depressed when LED is off.
	Reflection of light off of the DCU's glass capillary shaft.	Bring DCU up using the green knob.
3. DCU does not move by turning the green knob.	Calibration was set at minimum or maximum height.	Restart **UnipicK™** by powering off and on.
	DCU is too short and has reached its lowest position.	DCU may have been broken. Change to a new DCU.
4. Too much liquid accumulation in capillary shaft or filter is clogged.	Vacuum level and/or duration are set too high.	Decrease vacuum level and/or duration.
		Change the filter attachment.
5. Bubbles appear on the surface of tissue section.	The tissue section has been fixed before sectioning.	Make sure that the tissue is not fixed.
	Tissue sections are too thick.	Mount tissue sections with thickness of 50 µm or less.
6. Orange *Home* button does not light up.	The green knob was pressed repeatedly or *Home* button was pressed without bringing down the DCU at least 1mm.	Bring back the DCU to Starting position by pressing the blue *Retract* button or reset **UnipicK™** by powering off/on.

7. Orange *Home* button blinks continuously.	There is a leak in the vacuum line.	Contact NeuroInDx for inspection/repair.
	The movement of the DCU in the Sampler Head is obstructed.	Check to see if there is anything obstructing the movement of the DCU and remove it.

8 Technical Specifications

Specification	Description	
1. Illumination	Light source	144 LEDs ring light
	Input	24V DC
5. Min Dimensions	17.8 in/452 mm (L) x 17.2 in/437 mm (W) plus 126 mm (Control Box, W) x 15.2 in/386 mm (H)	
6. Max Dimensions	18.8 in/478 mm (L) x 21.1 in/537 mm (W) plus 126 mm (Control Box, W) x 22.1 in/561 mm (H)	
7. Max Weight Approx.	27.51 lb/12.5 kg	
6. Pump	Vacuum range	up to 22”Hg
	Input	24V DC
7. Linear actuator	Linear travel/step	0.0015 mm
	Maximum travel	23.3 mm
	Input	5V DC
8. Operating environment	Indoor use Altitude: max. 2000 meters Ambient temperature: 5°C to 40°C (41°F to 104°F) Maximum relative humidity: 80% for temperature up to 31°C (88°F) Supply voltage: 100VAC to 240 V AC, 50-60 Hz	

9 System Performance

Description	Specifications
Resolution	Single Cell
Vacuum duration (Ts), seconds	0.1 s to 1.0 s
Vacuum strength, Hg"	2.2" to 22" Hg
Available DCU IDs, µm	From 10 to100 µm
Acquisition speed (Hg"/Ts), seconds	
Minimum settings (4.4"/0.1 sec) Maximum settings (22.0"/1.0 sec)	1.3 s 2.2 s
Acquisition sample volume (Hg"/Ts/DCU ID) *	
DCU20 (µm) DCU30 (µm) DCU40 (µm)	10 nl to 2.5 µl 35 nl to 3.0 µl 70 nl to 5.0 µl
Cell collection speed (cells/minute) from tissue sections **	
Rat Purkinje cells (cerebellum) Mouse anterior horn motor neurons	12.0 ± 1.5 cells/min 12.0 ± 1.5 cells/min
Cell collection speed (cells/minute) from adherent cell cultures **	
SH-SY5Y human neuroblastoma cell line Chinese hamster ovary cells (CHO)	Up to 25 cells/min Up to 25 cells/min

* - - calculated for standard DMEM medium; acquisition volume depends on the DCU ID and sample viscosity

** -- estimated times are given as a reference and may be user/application-dependent

10 Warranty and Liability

NeuroInDx, Inc. warrants its products to be free of defects in materials and workmanship for a period of one (1) year from date of purchase unless otherwise specified at time of purchase. The foregoing warranty of NeuroInDx, Inc. shall be of no force and effect if buyer has modified or damaged the product. No other warranties of any kind, expressed or implied, are provided by NeuroInDx, Inc. NeuroInDx, Inc. shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products.

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SAFETY INFORMATION

Please read the instruction manual carefully in order to safely operate any NeuroInDx product

For Research Use Only. Not for use in diagnostic procedures.

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