

UNIPICK+

Cell and Tissue Acquisition/Deposition System with Universal Straddle Attachment

User Manual

Get a Hold of Your Cell



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1 Introduction

Unipick+[™], a capillary-based cell and tissue acquisition system, is the key to reliable, precise, 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 and deposition 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 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 can work with thicker tissue sections up to 500 µm, suitable for analyses that require large amounts of sample material, such as proteomics. **Unipick+**[™] may also be used for the collection of micrometric regions of interest from fixed (e.g. PFA-fixed) and archival tissue specimen, such as formalin fixed paraffin embedded (FFPE) tissue samples.

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, Next Generation Sequencing (NGS), 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+**[™].
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 any 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 and pressure 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+™** Sample Collection Assembly; (2) Vacuum and pressure Line and Electrical Cables; (3) Slider with micromanipulators; (4) Universal Microscope Straddle; (5) Front panel of **Unipick+™** Control Box with a touch screen; (6) Side panel of **Unipick+™** Control Box with a Homing button (top), Sample button (bottom) and a calibration wheel (green).

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 (2, 2.5, 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>

NOTE: The following installation of Universal Straddle is shown with Unipick™ model. The same sequence is used for Unipick+™ instrument.

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.



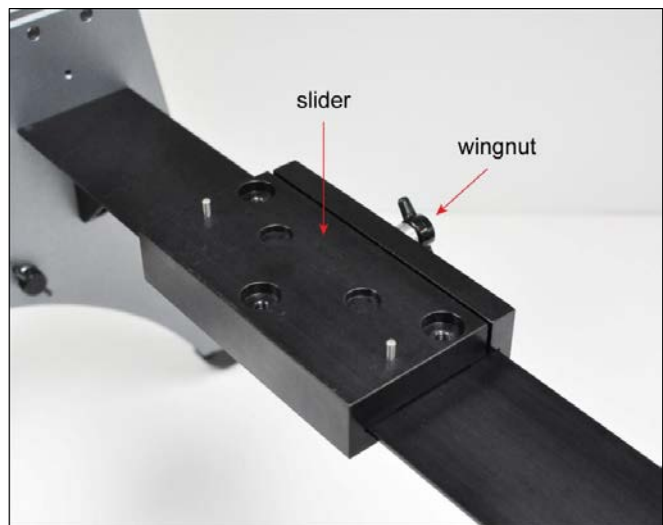
2. Assemble the Universal Straddle by attaching a bridge (short or long) to the curved legs with a pair of gussets and screws provided with the unit. DO NOT TIGHTEN THE SCREWS AT THIS TIME. There are two possible vertical positions where the bridge can be attached. Choose the appropriate height depending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



NOTE: Current assembly is shown with Unipick™, the same sequence is used for Unipick+™.

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. Use only the supplied power supply and cord.

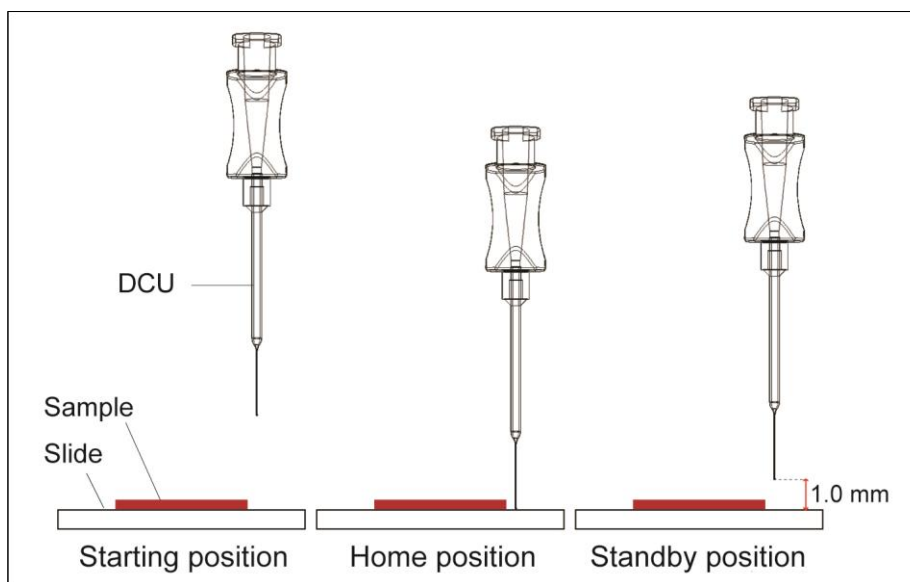
5 Using Unipick+™

5.1 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 or Dispensing position of the DCU tip determined by the user. Homing is performed prior to every collection/dissection and dispensing procedure.

Standby position: 1 mm above the Home position for **Acquisition mode**. DCU will be lifted to Standby position after each single acquisition or dispensing event. DCU returns to Standby position after each sampling until the Home position (Acquisition plane or Dispensing plane) or the system has been reset.



5.2 Power

To turn power on, turn the black switch on the back panel of the Control Box. At this point the screen and a green indicator light will turn on (**Figure 2**). To turn off the unit, press the switch again.

5.3 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.

5.4 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.

5.5 Positioning

To position the tip of the DCU along the z-axis, use the green calibration wheel (**Figure 2-5**) to move DCU up and down. A single click will move the DCU by 1.5 µm. When the wheel is moved fast, the DCU will move at a faster rate (default set at 150 µm/sec) specified in the **Settings window** of the **Unipick+™** software (please, see Section: **Unipick+™ Software**).

5.6 Home for the Acquisition and Dispensing planes

Use the green *Calibration* wheel (**Figure 2-5**) to set the Home position for *Acquisition* plane or *Dispensing* plane, i.e. the desired position of the DCU's tip on the z-axis for sample acquisition or dispensing. When the capillary tip comes in contact with the tissue surface or cells or desired dispensing plane (e.g. surface of the liquid in a sample tube), press the green calibration wheel (**Figure 2-5**). When the *Home* position for the acquisition mode is designated, the *Acquisition* plane icon will turn green and DCU will move 1 mm above that position on the z-axis to the *Standby* position. For the dispensing mode, the *Dispensing* plane icon will turn green and DCU will move to the *Starting* position.

5.7 Vacuum level and duration control

The vacuum or pressure levels may be controlled from 0 to maximum of 70 kPa and the impulse duration may be set from 100 ms to maximum of 1 s (see **Unipick+ Software** section). Depending on the sample and the desired result, various vacuum/pressure levels and impulse duration may be used. We recommend testing these parameters prior to any collection/dispensing procedure.

5.8 Sample

After the Home positions have been set, pressing the lower *Sample* button (**Figure 2-4**) will initiate sample collection or deposition by bringing the DCU down to the Home position and activating the vacuum or pressure impulse at the selected strength and duration.



Figure 2: *Unipick+™ control box includes: (1) Touch screen with controls; (2) Power indicator (green logo button); (3) Retract (upper blue button) to automatically moves 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 in acquisition mode; (4) Sample (lower green button): for cell/tissue collection and deposition; (5) DCU Positioning and Calibration (green wheel): to bring the DCU up or down towards the stage and set up Acquisition and/or Deposition planes.*

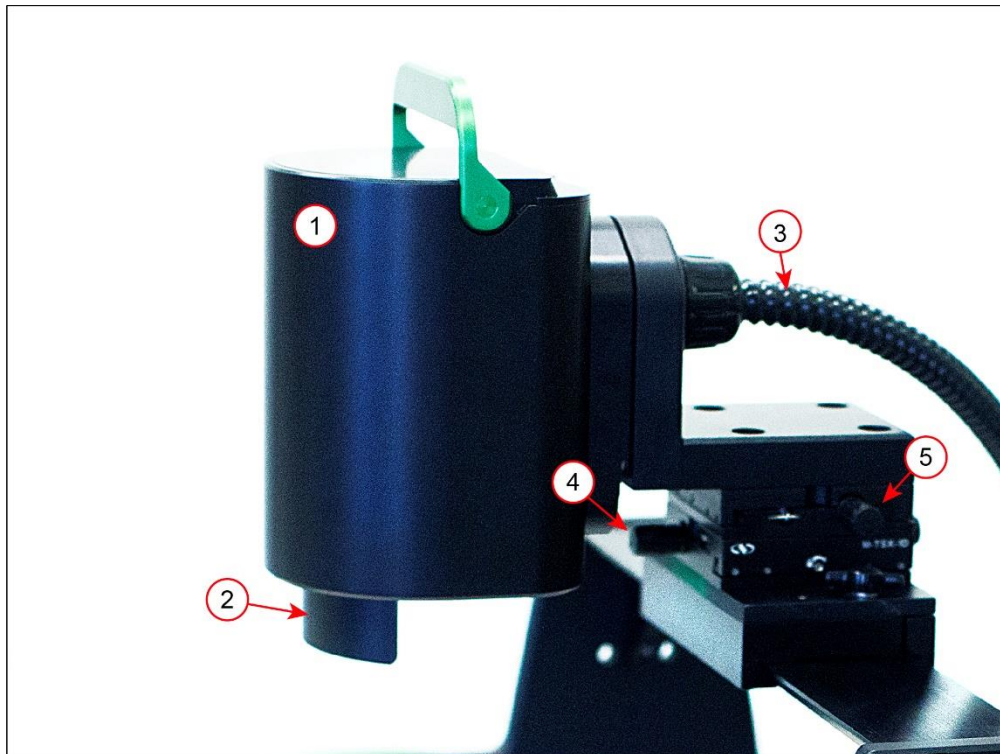


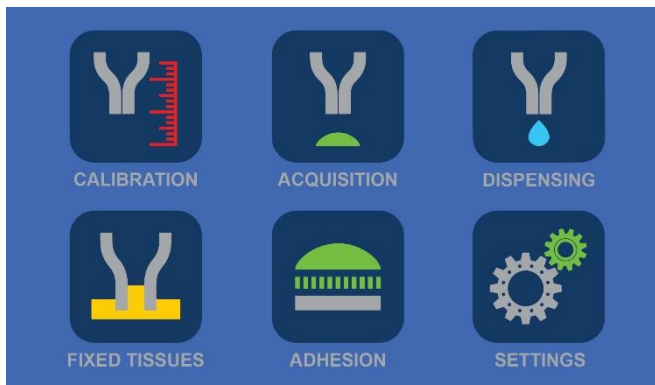
Figure 3: *Unipick+™* sample collection Head (1). The following components are shown: (2) Shield covering green horizontal calibration light used for DCU tip identification during calibration procedure; (3) Vacuum line and electrical cable. The Head should be turned full 90° for disposable capillary (DCU) attachment/removal. The x-y position of the DCU is controlled by the knobs on the linear stages/micromanipulators (4 and 5). The knob for micromanipulator on the front of the stage control the front to back movement of the DCU (4), and the one on the left side of the bottom stage control the side to side movement.

6 Unipick+™ Software - Main Controls

Unipick+™ software operates the instrument via the touch screen. It offers controls for calibration sequences, sample collection, dispensing, fixed tissue microdissection and single cell adhesion force measurements. The following section describes the main menus, operations, icons and their function. For further instructions on how to use these controls in a specific application, advance to “Initial Training Exercises with Unipick+™.”

6.1 Startup

Main menu is represented by six icons/options: Calibration – controls for setting stand by positions for both Acquisition and Deposition modes; Acquisition and Dispensing – controls for collection and sample deposition parameters, respectively; Fixed tissues – controls for working with fixed tissue specimen; and Settings – general controls of the **Unipick+™**;



6.2 Calibration

Left side of the menu has two sliding bars for the control of the main (left) and calibration (right) light intensities. Next to the sliding bars are controls for DCU's vertical movement (along z-axis). Single arrows apply slower motion (1000 $\mu\text{m}/\text{sec}$) and triple arrows move DCU in a fast mode (2,500 $\mu\text{m}/\text{sec}$). Internal diameter of DCU (from 10 to 100 μm) may be selected using the icons in the middle of the screen.



NOTE: Internal diameter of DCU must be selected if adhesion measurements are performed or the value of applied force is desired.

The following controls/icons are present on the right side of the **Calibration window**:



- Acquisition plane icon sets the plane where sample collection is to take place;



- Dispensing plane icon sets the plane where collected sample(s) will be deposited;



- The Retract feature remembers the most recent Home (*Acquisition* or *Dispensing* plane) position. It lifts the DCU to the Starting position and 15mm below the starting position in the Calibration Window. In Acquisition and Dispensing Windows, it will allow the user to move between Acquisition or Dispensing plane, Standby, and the initial Starting positions.

The icons on the right side of the window are found on all function windows to allow easy movement from one window to another. The icon of a window that is currently in use appears in grey.



- Home icon returns to the Main menu

6.3 Acquisition

The window offers controls for sample collection, including vacuum strength and impulse duration. It also keeps track of how many acquisitions were performed since the most recent calibration, time used, and force applied for each sample collection. Left side of the menu has the same sliding bars for light intensity control.



The following controls/icons are present on the right side of the **Acquisition window**:



- Collect icon is specific for the Acquisition window. It activates a single sample acquisition sequence.



- Green Calibration icon indicates that Calibration procedure (identification of Acquisition plane) was already performed



- Auto-dispensing icon takes you to the plate set up and auto-dispensing menu (Available for instruments equipped with a motorized stage. Please contact NeuroInDx representative for details.)



- When activated (turned green) this icon indicates that auto-dispensing process is ON (Available for instruments equipped with a motorized stage. Please contact NeuroInDx representative for details.)

NOTE: Acquisition plane may be changed by bringing the DCU to the desired location using the Green calibration wheel (Figure 2-5) then pressing the wheel. Same adjustments for the Dispensing plane may be performed directly within Dispensing window

6.4 Dispensing

The window offers controls for sample deposition including pressure strength, and impulse duration. Like **Acquisition window**, it keeps track of the number of dispensing events that were performed and the elapsed time since the most recent calibration. It also displays the force applied for each sample deposition. Left side of the menu has the same sliding bars for light intensity control, and the right side contains the main controls.



NOTE: We recommend using low pressure for dispensing starting from 2 kPa and longer duration time starting from 100 to 200 msec. Excessive pressure will result in a rapid sample dispensing spurting the DCU contents. If not sufficient, pressure and time duration may be adjusted for optimal sample deposition.

The following controls/icons are present on the right side of the **Dispensing window**:



- Dispense icon is specific for the Dispensing window. It activates a single sample dispensing sequence.

6.5 Fixed Tissues

This window provides manual and automatic pulse modes for acquisition of ROIs from fixed tissues such as formalin fixed paraffin embedded or PFA fixed tissue specimen. Pulse is an application of vacuum impulse with predefined duration and strength without lifting of the DCU tip from the tissue surface, which allows mixing the digestion liquid inside the capillary barrel.



The following controls/icons are present on the right side of the **Fixed Tissues window**:



- Manual pulse mode. Manual procedure initiates the collection sequence at any given time with possible incorporation of individual pulses as desired during the incubation (digestion) process.

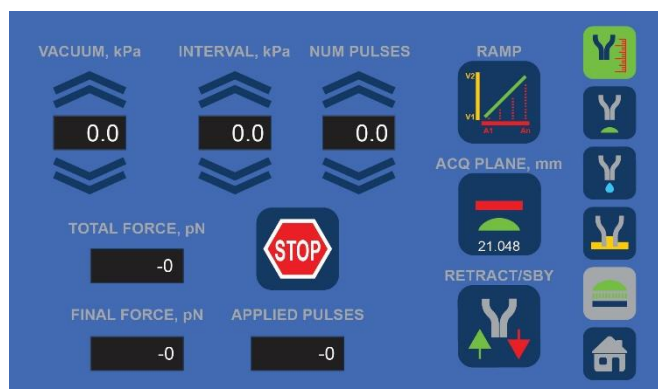


- Automatic pulse mode. For automatic process, incubation time and specific number of pulses during incubation period could be preset prior to initiation of a digestion procedure. At the end of digestion, the software initiates a standard acquisition sequence using predefined vacuum strength and impulse duration.

Collection procedure can be repeated for the next ROI using the same DCU or collected sample could be deposited in the desired sample tube using Unipick+ deposition mode.

6.6 Adhesion Strength

This function allows for the comparative estimation of single cell adhesion strength, e.g. how much force is required to lift a cell from the surface. Two output measurements are provided for the comparative analysis - “TOTAL FORCE” and “FINAL FORCE”. Both values are based on the algorithm of the RAMP function. TOTAL FORCE is defined as a sum of all forces corresponding to the total number of executed pulses. FINAL FORCE represents



the force used for the last and the highest vacuum pulse in the ramp process. APPLIED PULSES is the total number of pulses executed.

The following controls/icons are present on the right side of the **Adhesion window**:



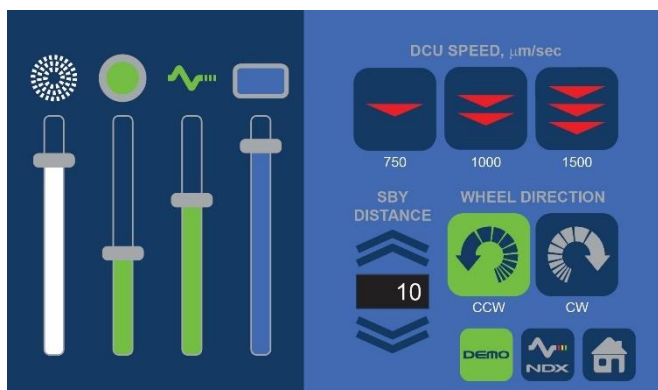
- Ramp function represents a specific number (NUM PULSES) of vacuum impulses starting with a predefined initial vacuum strength (VACUUM, kPa) raised by a predefined interval (INTERVAL, kPa) for each subsequent pulse.



- STOP icon may interrupt the RAMP process at any time. Current values for executed pulses (APPLIED PULSES), TOTAL FORCE and FINAL FORCE will be given.

6.7 Settings

Left half of the screen allows for the selection (from left to right) of main light, calibration light, NDX logo light, and screen light intensities. Three icons on the top right permit selection of DCU vertical movement speed for the fast mode of the calibration wheel.



Two icons on the lower right part of the screen offer a choice of the main calibration wheel direction to move DCU down: counterclockwise (CCW) and clockwise (CW) directions. The default distance for standby position during acquisition is 1mm from the acquisition plane; however, this can be changed to a shorter distance on this page.

The following controls/icons are present on the right side of the **Settings window**:



- CCW and CW icons permit selection of the main calibration wheel direction for moving DCU up or down



- Icons to select DCU movement speed

7 UnipickK+™ Software – Automation (optional)

Auto-collection and auto-dispense features allow the user to automatically collect and dispense multiple samples into PCR tubes. These features require a motorized stage (please contact NeuroIndx representatives for specifications). The process entails Plate setup (7.1), Dispensing parameters selection (7.2), and Target selection (7.3).

7.1 Plate set up

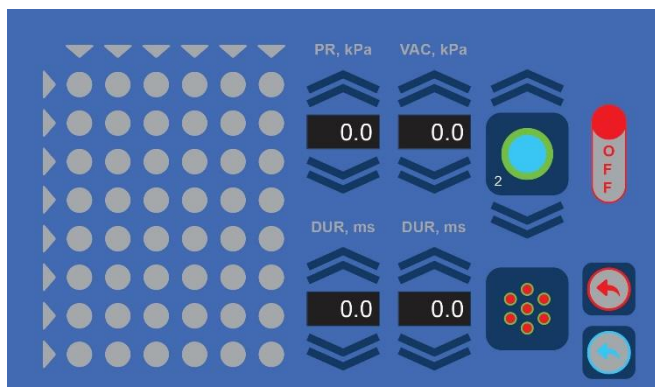
After initiating the auto dispensing icon (6.3), a plate set up and auto-dispensing window will appear. The left side of the window represents 48 well sample holder.

To set up a plate, move the automated stage to position the farthest back left well on the sample holder to align the center of the well with your DCU tip position on the x-y plane using the motorized stage. The top left corner well position on the window will be blinking. Tap the blinking top corner well position. The circle will stop blinking and turns dark blue when the stage position is calibrated.

Now move the stage to position the front most right well on the sample holder to align with your DCU position on the x-y plane. Tap the blinking bottom right well. The circle will stop blinking and change color to dark blue. Tap the OFF icon to ON. Plate calibration is complete (dark blue).

The collected samples will now be deposited from top left well to the bottom right well in a consecutive order.

Entire columns and rows may be deselected if necessary, by tapping on the corresponding triangle. For example, the image on the right shows that first four columns and a bottom row were



deselected, and only dark blue wells are remaining for depositing samples.

NOTE: Similarly, individual wells may be deselected (tapped once) or prioritized (tapped twice) for the deposition process.

NOTE: Before using the auto dispensing function, make sure to set the pressure on the Dispensing window at a minimum of 2kPa. However, the pressure applied for the deposition will be the value set on the Plate Set up/auto-dispensing window.

In addition to the plate set up, this menu offers options for capillary rinsing while depositing a sample. It permits multiple rounds of collection and dispensing within the same tube to ensure that DCU contents do not remain in the capillary. The number of such washing cycles is defined by tapping arrows above or below the blue dispensing icon on the top right of the window. Each tap corresponds to one wash cycle. The total number of cycles is shown in the lower left corner of the wash icon. Vacuum and pressure together with duration for washing cycle is set using the arrows in corresponding parameters.

The following controls/icons are present on the right side of the **Plate set up window**:



- Auto-collect screen icon. Tapping on this icon opens an **Auto-collect window**



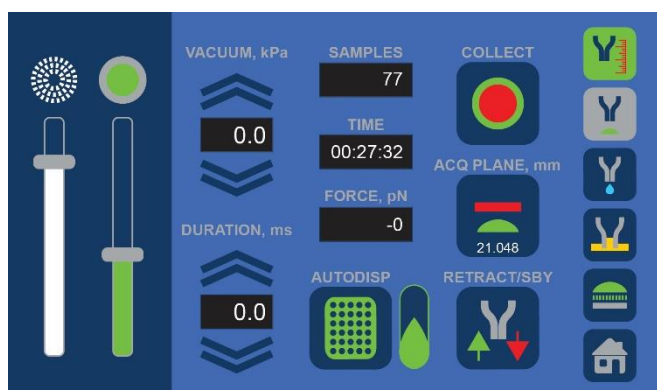
- Return to **Dispensing window**



- Return to **Acquisition window**

7.2 Auto-dispensing

Dispensing maybe directly initiated from the **Plate Set up/auto-dispensing window** using a Dispense icon. Alternatively, dispensing will occur automatically into designated location after each acquisition step if auto-dispensing droplet icon is activated. Note that AUTODISP icon is green indicating that the plate set up has been performed.



The progress of deposition will be shown in the auto-dispensing window that may be accessed any time by tapping the green AUTODISP icon. Wells where collected samples were deposited will be indicated with green. For example, the image on the right shows that already four samples were deposited in the first four wells. Four washing cycles were used for dispensing.



7.3 Auto-collection

Auto-collect screen icon opens the **Auto-collection window**, where up to eight targets may be preselected for automatic collection and deposition into designated locations (specified in the Auto-deposition window, 7.1). Acquisition parameters for selected targets are specified in the **Acquisition window** (6.3). Tapping on the AUTODISP icon would then open the **Plate set up/auto-dispensing window** with an Auto-collect icon.



Select either POOL or SINGLE for pooling collections into a single tube or singly collect and dispense (used for single cell collection). Move the stage to position the target in alignment with the DCU tip position on the x-y plane. Tap PRESELECT, and the position will populate the list under POSITION.

Tap the AUTODISP icon to go back to the **Auto-dispense window**. Tap the OFF icon on the right of the window to set to ON. Now the Auto-dispense function has been enabled and will turn green. Tap the red arrow icon on the window, which will take you to the **Acquisition window**. The Plate icon should appear in green. Tap the Eye drop icon next to it to activate the auto-dispense function. To start collection, tap the Collect icon. The system will begin its cycle and will collect eight preselected targets depositing them into designated locations with the wash cycle specific in the plate set up/auto-dispense window.

Make sure that the system has completed the full cycle and come to a complete stop (the stage should have returned to the last collection position). Tapping the ALL Red circle (icon) on the **Auto-collection window** will clear the previously selected positions.

The following controls/icons are present on the right side of the **Auto-collection window**:



- Preselect icon is used for selection of up to eight targets for auto-collection and deposition



- Pool icon selects “pool” mode of collection where up to eight preselected targets are collected and dispensed into a single tube



- Single icon selects “single” mode of collection where each target is collected and deposited separately into a separate tube. This mode is used for single cell collection.

8 Basic Operations

8.1 Initial Training Exercises with UnipickK+™

For instructional videos visit our website: www.neuroindx.com or YouTube channel: <http://www.youtube.com/NDXInc>

1. Start **UnipickK+™** by pressing the Switch on the back panel of the Control box once (green logo and a touch screen will light up).



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 connector on the **UnipickK+™** 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, this light may be turned off. It must be turned on again before lowering the DCU for calibration.

4. Rotate **UnipickK+™** Sampler Head clockwise so that the DCU tip points down.
5. Using the green *Calibration* wheel or DCU control arrows on the *Calibration window* menu, carefully bring the DCU down until the capillary tip touches the surface of the liquid. **Optional:** If the system has not been calibrated yet, press the *Retract* icon to bring the DCU tip down by 15 mm before using the *Calibration* wheel or touch screen controls.

NOTE: Single Calibration wheel movements (one increment) will move the capillary 1.5 µm per step. Moving Calibration wheel fast will increase the speed to the fast speed specified in the Settings window menu.

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 *Retract* icon to bring the DCU to its highest position and repeat the process from step 6.

8.2 DCU Calibration for Tissue Microdissection

Each new DCU must be calibrated before sampling. For instructional video visit our website: www.neuroindx.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 Switch on the back panel of the Control box once (green logo and a touch screen will light up). Place a tissue slide on the stage and add buffer to the tissue.
2. Position the slide to a clear spot on the slide adjacent to the tissue sample for calibration.
3. Rotate the **Unipick+**[™] Sampler Head 90° counterclockwise (**Figure 3**) to attach a filter directly to the male luer connector 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

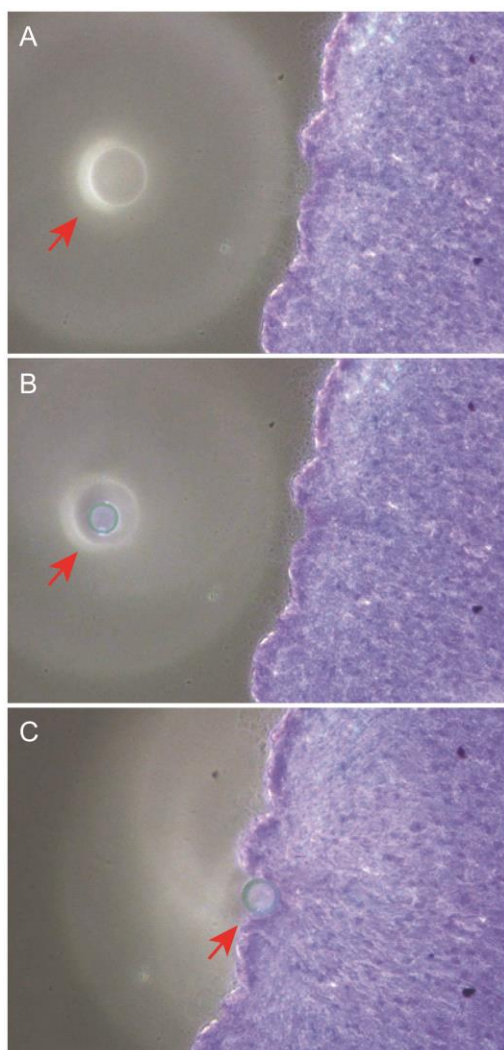
before lowering the DCU for calibration. The intensity may be adjusted using a sliding bar in the *Calibration* window menu]



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 UnipickK+'s mechanical parts. When UnipickK+™ is not in use, attach a new filter unit to prevent foreign objects from entering the system until next use.



4. Rotate **UnipickK+™** Head clockwise so that the DCU tip points down.

5. Using the green *Calibration* wheel or DCU control arrows on the *Calibration window* menu, carefully bring the DCU down until the capillary tip touches the surface of the liquid. **Optional:** Press the *Retract* icon to bring the DCU tip down by 15 mm first before using the *Calibration* wheel or touch screen controls.

NOTE: Quick button pressing will move the capillary 1.5 μm per step. Holding down the button for 5 seconds will increase the speed to 350 $\mu\text{m/s}$. Use caution.

6. 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.

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 XY mechanical stage to test if the DCU tip is in contact with the tissue section surface.*

7. 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 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, move DCU tip slightly up (~ few micrometers; one click of a green *Calibration* wheel corresponds to 1.5 μm) 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 microdissection.

8. Using the linear stages/micromanipulators to align the annulus of the capillary tip to the center of the ocular crosshairs.



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.

9. Press the *Acquisition plane* icon in the *Calibration* window to set the calibrated *Acquisition plane* or a *Home* position.
10. To re-calibrate, go to *Acquisition* window and press *Acquisition plane* icon. This will bring the DCU down to the calibrated *Acquisition plane*. Adjust the position of DCU with a green calibration wheel and once correct position is selected, press green calibration wheel once. This will set the new *Acquisition plane* and will lift the DCU to the *Standby position* 1 mm above the selected *Acquisition plane*.
11. You may re-calibrate at any time during the Acquisition procedure to designate a new Acquisition plane (Home) position.
12. Proceed to **Cell/Tissue Collection Section**

8.3 DCU Calibration for Adherent Cell Cultures

NOTE: Each new DCU must be calibrated before sampling. For instructional video visit our website: www.neuroindx.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. Do not add new medium in excess, the cells must be just barely covered.

1. Start **Unipick+**TM by pressing the Switch on the back panel of the Control box once (green logo and a touch screen will light up).
2. Place a cell plate/dish on the stage.
3. Rotate the **Unipick+**TM Head 90° counterclockwise (**Figure 3**) to attach a filter and DCU. Attach a filter directly to the male luer connector on the **Unipick+**TM 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+TM is not in use, attach a new filter unit to prevent foreign objects from entering until next use.

4. Rotate **Unipick+**TM Head clockwise so that the DCU tip points down.
5. Using the green *Calibration* wheel or DCU control arrows on the *Calibration window* menu, carefully bring the DCU down until the capillary tip touches the surface of the liquid. **Optional:** If the system has not been calibrated yet, press the *Retract* icon to bring the DCU tip down by 15 mm before using the *Calibration* wheel or touch screen controls.

NOTE: Single Calibration wheel movements (one increment) will move the capillary 1.5 µm per step. Moving Calibration wheel fast will increase the speed to the fast speed specified in the Settings window menu.

6. Use the linear stages/micromanipulators (**Figure 3**) to locate the DCU halo.
7. 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 contacted 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 (e.g. **Figure 4**).
8. 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, move DCU slightly up 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.
9. 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.

10. Press the *Acquisition plane* icon to set the calibrated *Acquisition plane* or Home position.
11. Move to *Acquisition* window and press a green Calibration wheel. The DCU will be lifted to the Standby position 1 mm above the sampling position. At this point the instrument is prepared to collect cells.
12. To re-calibrate, from *Acquisition* window simply press the *Acquisition plane* icon to bring the DCU back to Home position. Re-position the capillary tip using the up and down arrows or by rotating a *Calibration wheel* and then push the *Calibration wheel* again to set the new Home position.
13. You may re-calibrate at any time during the sampling procedure to designate a new *Acquisition plane* (Home) position.
14. Press *Retract* icon and make sure that DCU is in the UP position. Move microscope X-Y stage to the position where the sample needs to be dispensed.
15. Calibrate DCU to identify *Dispensing plane* and set the Home position for the dispensing using the same sequence as described for the selection of *Acquisition plane*.
16. Proceed to **Cell/Tissue Collection Section**



Caution: When the UnipickK+™ 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.

8.4 Cell and Tissue Collection

1. Make sure *Acquisition* window is selected
2. 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.

3. 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.
4. Press the *Collect* icon or a green sample button on the side of the control box to acquire the targeted cell/tissue.
5. 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.**
6. Press *Retract* icon to move DCU in the upward position.
7. Move to the Dispensing window. Moving the microscope X-Y stage identify the area where the sample must be dispensed.
8. Adjust pressure and duration values. We recommend starting from 10 kPa/10 msec and gradually increase vacuum force if necessary. In some cases, longer impulse (time duration) may help acquisition.
9. Press *Dispense* icon to release the DCU contents. This will bring the DCU down to the Dispensing plane and will initiate a single dispensing event. If the sample did not leave the DCU entirely, activate Dispensing icon again until the DCU contents are deposited in the desirable location.
10. Push the *Retract* icon to bring the DCU in the upward position.
11. Change to the *Acquisition* window, move the X-Y stage to the desired collection position and repeat the procedure for the next sample collection.

NOTE: DCU contents may also be released using a syringe with a male luer lock. 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. Attach a DCU to Unipick+™ Head and re-calibrate to continue collection.

8.5 Dispensing DCU Contents

Option 1: This option is only available when both *Acquisition* and *Dispensing* Planes are the same

1. Once a sample is collected, in the *Acquisition window*, press *Acquisition Plane* icon to move DCU to the current *Acquisition* plane position.
2. Switch to *Calibration window*. Press *Dispensing Plane* that will set the *Dispensing* position.
3. Go to the *Dispensing window*. Press *Retract* (blue illuminating button on side of a control box or *Retract/Standby* icon) twice to move DCU to *Starting* position. Move microscope stage to adjust the location at which the DCU will dispense.
4. Set *Pressure* and *Duration* parameters.
5. Once location and parameters are set, press *Dispense*. The DCU will move down to selected *Dispensing Plane* and *Dispense* its contents.
6. If DCU is not fully emptied, adjust *Pressure* and *Duration*. Repeat the *Dispense* procedure.

Option 2:

1. Once sample is collected, press the illuminating blue *Retract* button on the side of the control box or *Retract/Standby* icon in the *Acquisition* menu. This will move DCU to the *Starting* position.
2. Adjust microscope stage to identify location where the sample must be dispensed.
3. Use green *Calibration* wheel or arrows in the *Calibration window* to lower the DCU until desired *Dispensing plane* is reached. Press *Dispensing Plane* icon to set the new *Dispensing Plane* position.
4. Go to the *Dispensing window*. Set *Pressure* and *Duration* parameters. Press *Dispense*.
5. If the DCU is not fully emptied, adjust *Pressure* and *Duration*. Press *Dispense*.

6. Repeat the procedure until the DCU is emptied.

8.6 Using Retract Function to Move Between Wells

1. Press the *Retract* icon in the *Acquisition* window. 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 *Retract* icon again to return the DCU to its Standby position.
4. Press the *Acquisition* plane icon from Standby position to bring the DCU down 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 arrows.

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

5. Press the Green calibration wheel to designate the new *Acquisition* plane. The DCU will move up to the new Standby position.
6. Resume collection.

8.7 Manual Dispensing of DCU Contents and Reusing the Same DCU:

1. Press the *Retract* icon from the screen or the blue illuminating *Retract* button on the side of the control box (**Figure 2-3**). This will bring the DCU up to its original *Starting* position.
2. Rotate the **Unipick+**[™] Head 90° counterclockwise to access the DCU.
3. Press the *Retract* icon or *Retract* blue illuminating button again to bring the DCU out for easier access.
4. Remove the DCU and dispense contents if necessary using a syringe as previously described (**Figure 5**).
5. Attach the DCU to the filter on the Head and press the *Retract* button or icon. 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 *Retract* icon in the *Calibration* menu; this will bring the DCU down to the *Standby* position. **Do not press Acquisition plane icon.** The actual DCU tip position may have been changed due to the detachment/attachment procedure. It is safer to recalibrate the DCU.
8. Align the annulus of the capillary tip to the center of the ocular crosshairs or a screen using the linear stages/micromanipulators and/or adjust the z-axis position of the DCU using the green wheel on the side of the control box (**Figure 2-3**) or DCU control arrows displayed on the *Calibration Window*.
9. Set the new *Acquisition* position, change to *Acquisition Window* and push the green calibration wheel. Now, the sample button on the side of the control box will illuminate green indicating that the home position has been set. The DCU will lift to the *Standby* position 1 mm above the *Acquisition* position.
10. Resume collection.

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.

8.8 Changing DCUs

1. Press the blue illumination *Retract* button on the side of the control box (**Figure 2-3**) or a *Retract* icon in any of the menus 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 or icon 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 or icon. 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 *Retract* button or icon again to bring the DCU down to the most recently set *Standby* position.
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 using the green *Calibration* wheel to determine the new Home position.
9. Press the green *Calibration* wheel to designate the new *Acquisition* plane or *Home* position. The DCU will move up to the new *Standby* position.
10. Perform the same procedure if the *Dispensing* plane needs to be set.
11. Resume collection.

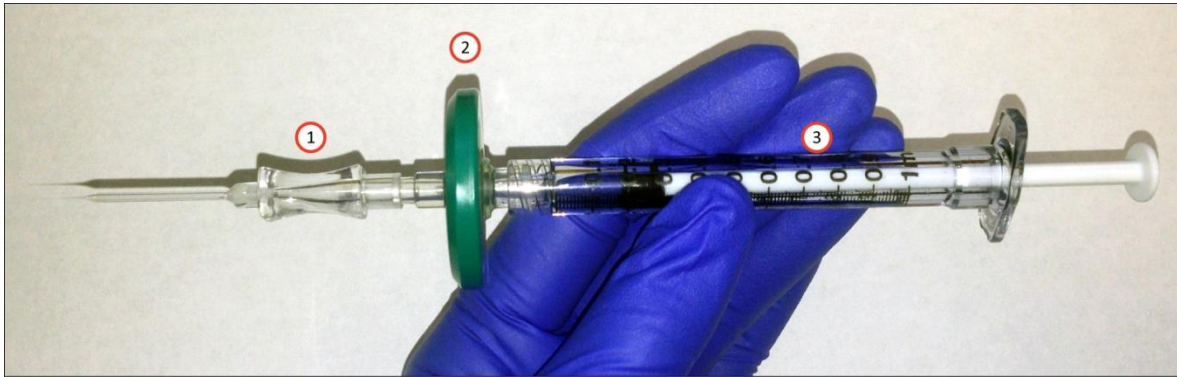


Figure 5: *DCU and filter affixed to a Luer lock sterile syringe. 1 – DCU; 2 – filter, 3 – syringe.*

9 Sample Protocols

Protocol 1: Collection of Individual Adherent Cells from Culture Dishes

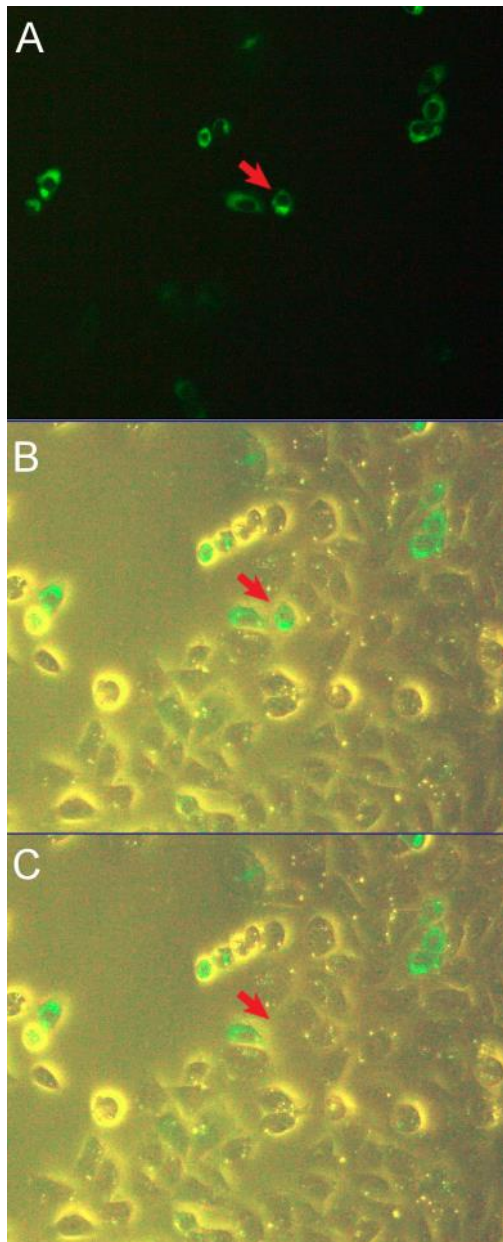
Various adherent cell cultures including human neuroblastoma cell line SH-SY5Y, Chinese hamster ovary (CHO) cells, human melanoma MDA-MB-435 cells and various primary cultures, such as neural progenitors, skin fibroblasts, etc., can be used for the collection of individual cells using both **Unipick™** and **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.neuroindx.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% CO₂ 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 *Collect* icon in the *Acquisition* window. 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, 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: Follow the instructions for Dispensing the DCU Contents

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 preperfusion 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.neuroindx.com/Unipick+/application-notes/>

Microdissection or Single Cell Collection:

Dry the back of the slide after PBS wash and place the slide on 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.

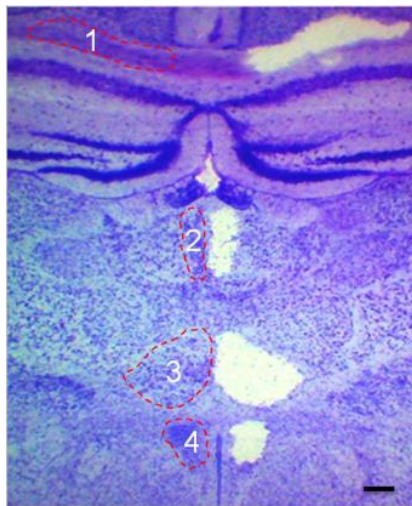


Figure 7. Unilateral microdissection of the corpus callosum (1), paratenial thalamic nucleus (2), nucleus reunions (3) and hypothalamic nucleus (4). Tissue was stained with toluidine blue. Tissue thickness 50 μm. Scale bar= 250μm

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 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.

Ejecting Cell/Tissue from DCU:

Follow the instructions for **Dispensing the DCU Contents**. Alternatively, use the following manual protocol: Prior to removing a DCU from **Unipick+**TM Head, prepare a syringe by pulling the syringe plunger back and attaching a filter to the syringe tip (**Figure 5**). Then, from the *Standby* position, press the blue illuminating *Retract* button on the side of the control box (**Figure 2-3**) or the *Retract/Standby* icon. Carefully remove the DCU and attach it to a syringe. Carefully position the DCU tip into preloaded buffer and slowly eject cell/tissue sample 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 or other desired buffer 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

Both **Unipick™** and **Unipick+™** can 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 hours.¹ See Application Notes on our website: <http://www.neuroindx.com/Unipick+/application-notes/>

Materials:

- Vibratome
- Thermostat bath
- Surgical instruments
- Artificial Cerebrospinal Fluid (ACSF) 300-500ml containing (in mM): 126 NaCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 2.5 KCl, 2 CaCl₂, 2 MgCl₂, and 10 D-glucose, pH 7.4, carbogen (95% O₂ 5% CO₂). 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 another anesthetic approved by your ARC.
3. Open the chest and flush the animal through the aorta with cold aerated ACSF for 3-5 minutes.

² Practical Electrophysiological Methods: A Guide for In Vitro Studies in Vertebrate Neurobiology. (1992) Eds. [Helmut Kettenmann](#) and [Rosemarie Grantyn](#), Wiley-Liss, New York

4. Excise the brain, rinse with cold ACSF, and remove excess liquid with sterile napkin.
5. Keep brain in buffer consisting of Krebs-Ringer solution saturated with carbogen (95% O₂, 5% CO₂) 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 a 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.neuroindx.com or YouTube channel: <http://www.youtube.com/user/NDXInc>

Ejecting Cell/Tissue from DCU:

9. Prior to removing a DCU from **Unipick+**TM Head, pull the syringe plunger back and attach a filter to the syringe tip.
10. Carefully remove the DCU from **Unipick+**TM 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% CO₂ 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 CaCl₂
- 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

Prepare on the day of dissection

1. **Dissociation Solution** by mixing the following components to make 10ml of solution
 - a. 200µl Liberase stock solution
 - b. 75µl 1M CaCl₂
 - 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 another 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+**TM 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.neuroindx.com or YouTube channel: <http://www.youtube.com/user/NDXInc>

Ejecting Cell/Tissue from DCU:

12. Prior to removing a DCU from **UnipickK+**TM Head, pull the syringe plunger back and attach a filter to the syringe tip.
13. Carefully remove the DCU from **UnipickK+**TM 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.

10 Troubleshooting

If problems with **UnipickK+™** 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 UnipickK+™ Head.	Turn the green LED off.
	Reflection of light of the DCU's glass capillary shaft.	Bring DCU up with using arrows or a green <i>Calibration</i> wheel.
3. DCU does not move	Calibration was set at minimum or maximum height.	Restart UnipickK+™ 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.

11 Technical Specifications

Specification	Description	
1. Illumination	Light source	122 LEDs ring light
2. Min Dimensions	17.8 in/452 mm (L) x 17.2 in/437 mm (W) plus 9.3 in/237 mm (Control Box, W) x 24.7in/626 mm (H)	
3. Max Dimensions	18.8 in/478 mm (L) x 21.1 in/537 mm (W) plus 9.3 in/237 mm (Control Box, W) x 24.7in/626 mm (H)	
4. Max Weight Approx.	38.5 lb/17.5 kg	
5. Pump	Vacuum range	up to 70 kPa
	Pressure range	Up to 36 kPa
	Input	24V DC
6. Linear actuator	Linear travel/step	0.0015 mm
	Maximum travel	26.5 mm
	Input	5V DC
7. 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	

12 System Performance

Description	Specifications
Resolution	Single Cell
Vacuum duration (Ts), seconds	0.1 s to 1.0 s
Vacuum strength, kPa	Up to 70 kPa
Pressure strength, kPa	Up to 36 kPa
Available DCU IDs, μm	From 10 to 100 μm
Acquisition/Dispensing speed (kPa/Ts), seconds	
maximum settings (0.1 sec)	1.3 s
Maximum settings (1.0 sec)	2.2 s
Acquisition sample volume (kPa/Ts/DCU ID) *	
DCU20 (μm)	10 nl to 2.5 μl
DCU30 (μm)	35 nl to 3.0 μl
DCU40 (μm)	70 nl to 5.0 μl
Cell collection speed (cells/minute) from tissue sections **	
Rat Purkinje cells (cerebellum)	12.0 ± 1.5 cells/min
Mouse anterior horn motor neurons	12.0 ± 1.5 cells/min
Cell collection speed (cells/minute) from adherent cell cultures **	
SH-SY5Y human neuroblastoma cell line	Up to 25 cells/min
Chinese hamster ovary cells (CHO)	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

13 Warranty and Liability

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14 Contact

NeuroInDx, Inc.
20725 S Western Ave, Ste 100
Torrance, CA 90501
USA
Tel. +1-424-731-7512
Fax. +1-424-731-7514
info@neuroindx.com
www.neuroindx.com

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NeuroInDx, Inc.

20725 S WESTERN AVE, STE 100
Torrance, CA 90501-1885

SAFETY INFORMATION

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

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CONTACT US

www.neuroindx.com

info@neuroindx.com

Tel: 1 424 731 7512

Fax: 1 424 731 7514