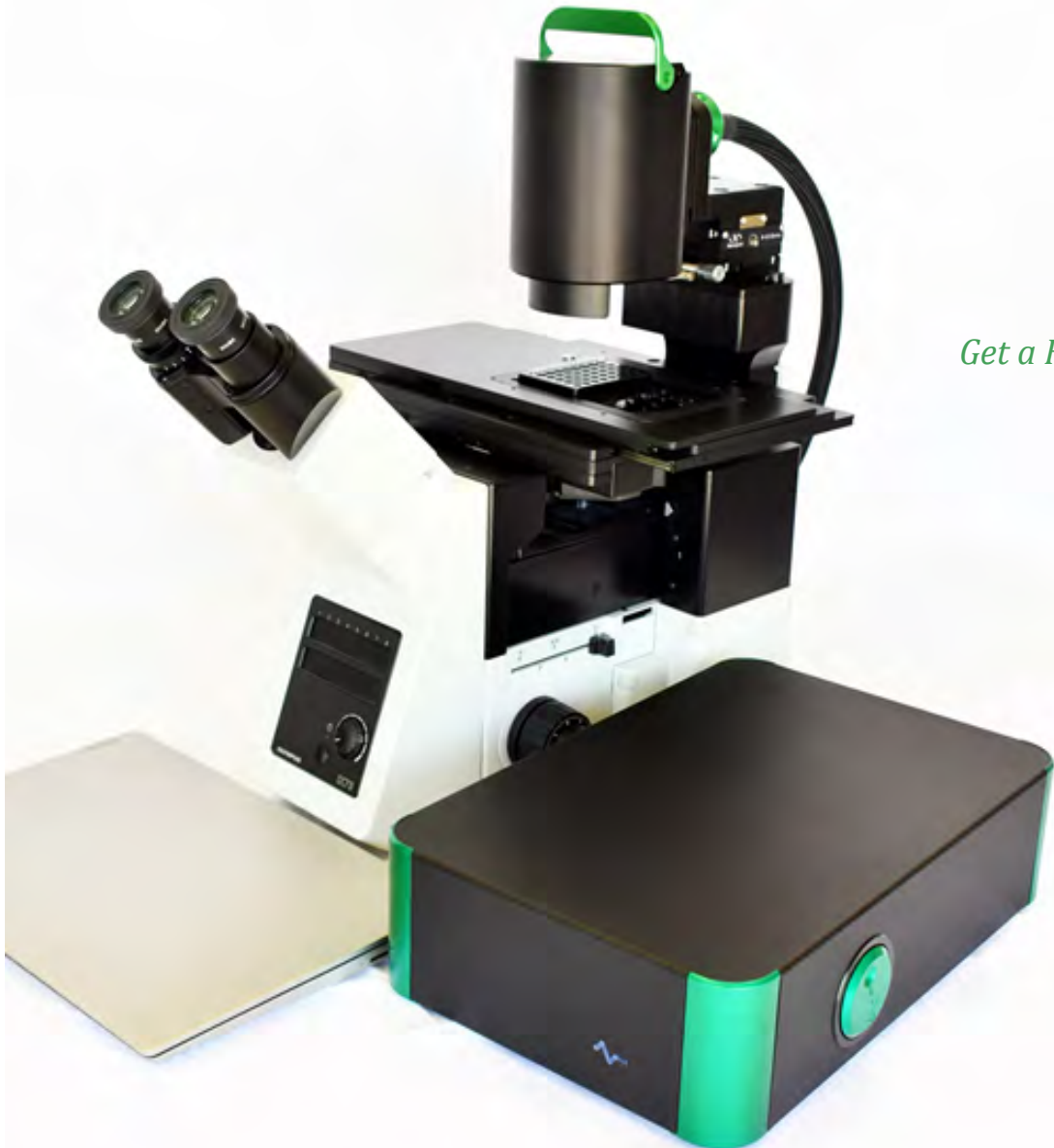


A-PICK

Automated Cell and Tissue Acquisition/Deposition System

User Manual



Get a Hold of Your Cell

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1 Introduction

A-pick™, an automated capillary-based vacuum pulse assisted 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 pulse-assisted capillary based system, **A-pick™**, is compatible for direct integration with Olympus IX51/71/81 and IX73/83 inverted microscopes or for use with Olympus IX53, NIKON Eclipse MA100, and other inverted microscopes (check for compatibility) using the universal microscope straddle (sold separately).

It allows for automated 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. **A-pick™** 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, **A-pick™** 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.

A-pick™ can work with tissue sections up to 500 µm thickness, suitable for analyses that require large amounts of sample material, such as proteomics. **A-pick™** 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 **A-pick™** 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 **A-pick™** over an inverted microscope, level surface and allow at least 5 inches of space all around **A-pick™**.
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 **A-pick™**, route the power cord away from the microscope frame and **A-pick™** unit.
5. Always ensure that the grounding terminal of the **A-pick™** 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 **A-pick™** 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 **A-pick™** is not in operation, be sure to turn the power off and disconnect the power cord from the connector socket of the **A-pick™** unit or from the wall power outlet.
9. Dispose used capillaries (DCUs) into a biohazard glass waste container according to regulations.



Caution: **A-pick™** components contain hazardous materials. **A-pick™** consumables should be disposed according to local regulations.

3 Components and Accessories

A-picK™ (Figure 1) consists of Sample Collection Assembly (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. **A-picK™** may be purchased with a direct adapter or a Universal Straddle that fits over an inverted microscope with a SCAN IM motorized stage from Märzhäuser Wetzlar GmbH & Co.KG. Ready-to-use Disposable Capillary Units (DCUs) for cell collection and tissue dissection and filter attachments are sold separately.



Figure 1: Major components of **A-picK™**: (1) **A-picK™** Sample Collection Assembly (Head); (2) Micromanipulators; (3) Vacuum and Pressure Line and Electrical Cables; (4) Motorized stage; (5) Universal Sample Holder; (6) Olympus IX73 Inverted Microscope; (7) Personal computer with **PIKCELLS™** software for process control; (8) **A-picK™** Control Box; (9) Side panel of **A-picK™** Control Box with the green Calibration wheel.

4 Initial Set Up

Make sure that the following items are present for proper operation of **A-picK™**:

1. **A-picK™** unit (Sample Collection Assembly and Control Box) (Fig. 1) - supplied
2. Inverted Microscope compatible with **A-picK™** (Fig. 1)
3. Motorized microscope stage (Märzhäuser Wetzlar GmbH & Co.KG SCAN IM) (Fig. 1)
4. 0.5X C-mount adapter for camera
5. Hex wrenches (2, 2.5, 3, 4, & 5 mm) - supplied
6. Power supply and cord (one of each) - supplied
7. Personal computer with mouse and keyboard meeting the following requirements.
 - a. Intel i5/Ryzen 3 or better
 - b. 8GB RAM or more
 - c. SSD Drive
 - d. USB3

Proper installation of all parts is required for **A-picK™** 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.

NOTE: Sample videos and straddle installation instruction video are available on our YouTube channel: <http://www.youtube.com/NDXInc>

NOTE: If Universal Straddle is used instead of direct integration with a microscope, please refer to UnipicK+™ instruction manual at www.ndx-instruments.com/support/downloads. The same installation sequence is used for A-picK™ instrument utilizing the Universal Straddle system.

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 **A-picK™** control box and plug the power cable into the outlet. Use only the supplied power supply and cord.

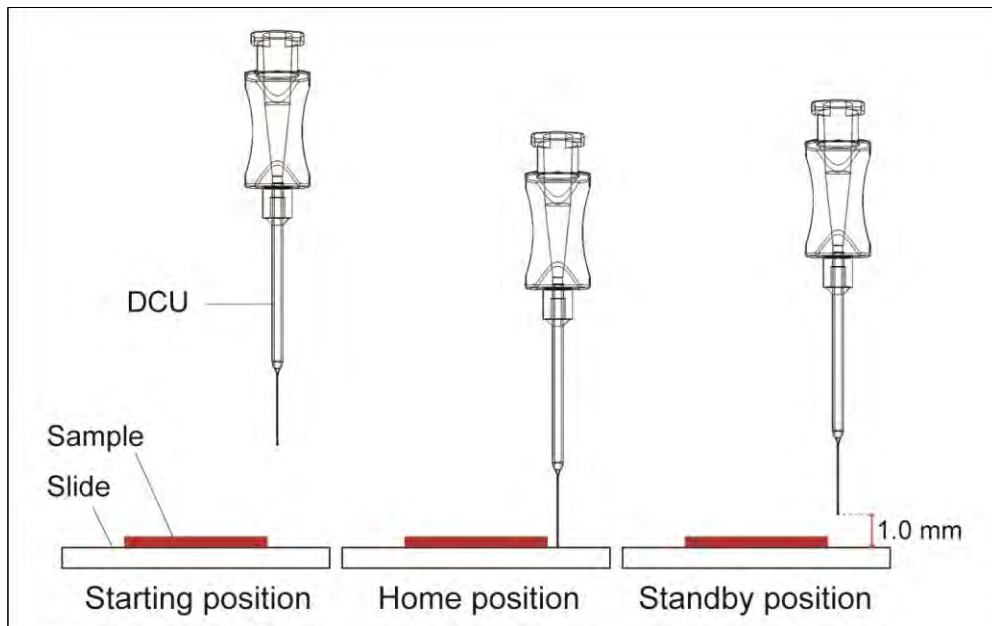
5 Using A-pick™

5.1 Terminology

Starting position: Highest vertical position for the DCU (i.e. 0); the initial position when the unit is first turned.

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 (recommended) above the *Home* position for **Acquisition mode** and 10 mm for **Dispense 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 Dispense plane) or the system has been reset.



5.2 Power

To turn the power on, turn the black switch on the back panel of the Control Box. The green indicator light on the back will turn on (**Figure 1**). To turn off the unit, press the switch again.



Caution: Do not switch the system on and off quickly. After turning the system off, wait at least 10 seconds before switching it back on

5.3 Filter and DCU attachment

Turn the **A-picK™** Head 90° counterclockwise to expose the male luer connector. Holding the outer rim of the filter, connect the female luer lock connector of the filter to the male luer connector on the **A-picK™** 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.

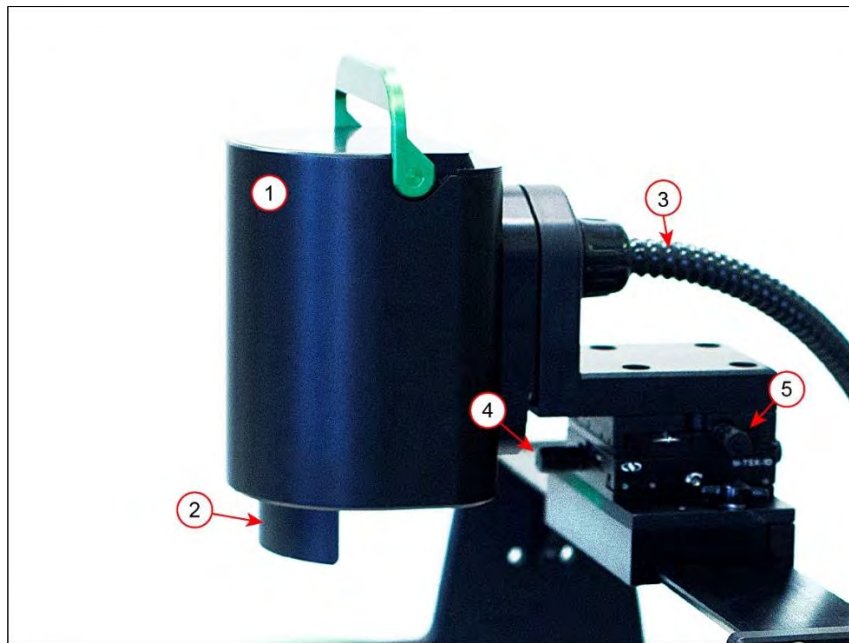


Figure 2: **A-picK™** Sample Collection Assembly, a.k.a. Head (1). The following components are shown: (2) Shield covering the green horizontal calibration light used for DCU tip identification during calibration procedure; (3) Vacuum line and electrical cable. The Head should be turned full 90° for 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).

5.4 X-Y control

To center the tip of the DCU in the field of view, use the manual dials (**Figure 2**) on the linear stages/micromanipulators at the base of the **A-picK™** Head at the mounting site.

6 PIKCELLS™ - Main Controls and Operation

PIKCELLS™ software operates the instrument via personal computer. It offers controls for calibration sequences, plate setup, sample collection, sample dispensing, fixed tissue microdissection, single cell adhesion force measurements, and process inspection and documentation. The following section describes the main menu, operations, icons and their function. For further instructions on how to use these controls for a specific application, advance to “Initial Training Exercises with **A-pick™**.”

6.1 MAIN MENU

Main menu is represented by six icons/options:

CALIBRATION controls for setting positions for both Acquisition and Deposition planes. It identified and autocorrects the DCU tip position relative to the X-Y stage and measures the DCU tip internal diameter, which is later used for calculating the applied force.

The icon turns green when both acquisition and deposition planes are set.

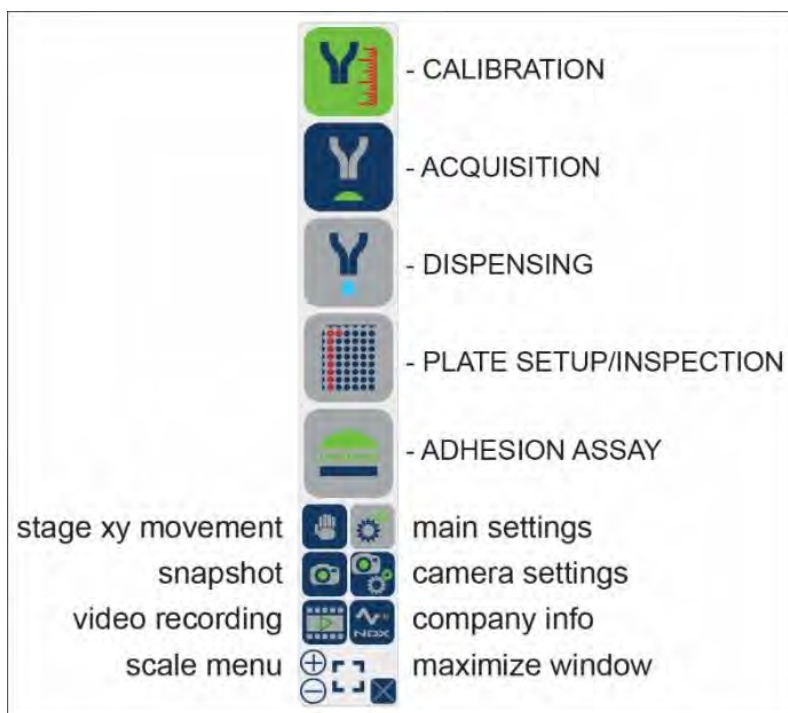
ACQUISITION and **DISPENSING** control collection and sample deposition parameters, respectively.

PLATE SETUP/INSPECTION defines deposition sequence and designation of wells. It also permits for inspection of a collection process after acquisition/deposition sequence is complete.

ADHESION ASSAY offers controls for measuring single cell adhesion or detachment force using RAMP assay or manual measurements.

SETTINGS window provides general controls of **A-pick™**, including light intensity, DCU’s vertical speed, calibration wheel direction, and Standby and Retract distances.

Remaining **MAIN MENU** icons in the lower part of the menu offer X-Y stage movement, camera controls, snapshot and video taking. The user may also regulate the size of the menu icons for convenience.



6.2 CALIBRATION

The arrows on the upper right-side (**Z-AXIS**) control the DCU's vertical movement along the z-axis. The mouse icon at the top right allows for switching the controls of the z-axis movement to a computer mouse wheel. Single click of an arrow or a mouse wheel corresponds to a single 1.5 μm movement. Desired distance may be also typed in the space between the arrows.

Keep in mind that vertical z-axis movement may be also performed with the green calibration wheel found on the right side of the control box. Similarly, a single click of a slight rotation will move the DCU by 1.5 μm . When the wheel is turned fast, the DCU will move at a faster rate (1000 $\mu\text{m}/\text{sec}$).

ACQ, μm is an acquisition plane icon that sets the plane where sample collection is to take place, i.e. where the DCU tip meets the sample. It is found by slowly lowering the DCU with one of the methods described above and finding a plane where both DCU tip and the sample to be collected are in the same focus. This may be done using microscope eye pieces or observing via computer monitor. When the capillary tip comes in contact with the tissue surface or cells, click on the **ACQ, μm** icon. When the acquisition plane is designated, the **ACQ, μm** icon will turn green. Detailed instructions with initial training will be provided during installation.

DISP, μm is a dispense plane icon that sets the plane where collected sample(s) will be deposited. It may be identified in a similar fashion by lowering the DCU to a desired plane (e.g. surface of the liquid in a sample tube) or preset using one of the **ACQ MODE** icons found on the right side of the menu if one of the standard collection scenarios is used. **DISP, μm** icon will turn green after dispense plane is identified.

ACQ MODE icons offer one of the four common scenarios: collection from slide to a standard 100 ml PCR tube with 20 μl or 50 μl liquid (left) or collection from 3.5 cm Petri dish to a standard 100 ml PCR tube with 20 μl or 50 μl liquid (right). Choosing one of the four acquisition modes will automatically set the dispense plane – **DISP, μm** icon will turn green and a numerical value will be shown under the icon.

NOTE: NeuroInDx's universal sample holder must be in place to use predefined acquisition/dispensing modes.

RET/SBY icon quickly raises or lowers the DCU to predefined positions preset in the **SETTINGS** window (see **SETTINGS** section).



FIND XY icon automatically calibrates the live image distance relative to the movement of the X-Y stage. This is used for automatically finding and sizing the DCU, and is necessary in order to use the Auto Tip Positioning function.

AUTO and **TUNE** icons automatically measure the DCU's internal and external diameters to calculate force applied for lifting a target cell, as well as allow for adjustment override by the user.

6.3 ACQUISITION

The window offers controls for sample collection, including vacuum strength, impulse duration, target selection, collection mode and acquisition of regions of interests from fixed tissues or hard to dissect tissues. It also keeps track of how many acquisitions were performed since the most recent calibration.

VAC, kPa and **DUR, ms** arrows at the top provide controls for vacuum strength and duration. The vacuum level may be controlled from 0 to maximum of 70 kPa and the impulse duration may be set from 1 ms to maximum of 1000 ms. Depending on the sample and the desired result, various vacuum levels and impulse duration may be used. We recommend testing these parameters prior to any collection/dispensing procedure. We recommend starting with low values and gradually increasing them until the target sample is successfully lifted.

SELECT icon is used to select desired targets (cells or tissue regions). Selection is performed via computer screen and a mouse by clicking on a desired target. Multiple targets may be selected for a single run.

Two icons below **SELECT** icon (**POOL** and **SINGLE**) are used to designate a specific collection-deposition mode. When the **POOL** icon is activated multiple targets are selected and dispensed into a single tube.

Selecting the **SINGLE** icon activates the mode of collection in which each selected target is collected and deposited into a separate tube. This mode is used for single cell collection.

There are two icons in the middle of the **ACQUISITION** window. **ABORT** icon stops and cancels the entire process. **ACQ PLN** icon indicates the current acquisition plane and may be used for its slight adjustment (recalibration) by bringing DCU to the current acquisition plane (e.g. 19000 μm) and then using the **CALIBRATION** window controls to adjust the vertical position of the DCU tip.



After the targets are selected, clicking on the **GO!** icon at the lower right corner will initiate sample collection and deposition by bringing the DCU down to the acquisition plane and activating the vacuum and to the dispense plane and activating a pressure impulse at the selected strength and duration.

The two lower icons (**PULSE** and **MANUAL**) and arrows above them provide automatic and manual pulse modes for acquisition of regions of interest (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.

PULSE icon provides a mode when DCU is in touch with a tissue surface and remains in touch for the incubation time (in seconds) specified using the arrows above. **PULSE** icon turns green when activated. For automatic process, incubation time (**INCBTS, S**) and specific number of pulses (**PULSES**) during incubation period could be preset prior to initiation of a digestion procedure. At the end of digestion, a standard acquisition sequence is automatically initiated using predefined vacuum strength and impulse duration.

6.4 DISPENSING

The window offers controls for sample deposition including desired washing sequence. Like **ACQUISITION** window, it keeps track of the number of dispensing events that were performed (lower right corner).

PRESS, kPa and **DUR, ms** at the top offer settings for pressure strength and impulse duration, respectively.

ABORT icon stops the entire deposition sequence.

DISP PLN icon brings the DCU tip to the plane where sample dispensing is set. This icon may be used for quick adjustment of the dispense plane during the collection/deposition sequence.

DISPENSE icon activates a single sample dispensing sequence.

Three lower arrow settings provide controls for sample rinsing to ensure all collected material leaves the capillary. These controls are not required but may be useful when extra small amounts of material are collected and dispensed.



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.

WASH icon at the bottom left of the **DISPENSE** window activates capillary rinsing that permits multiple rounds of collection and dispensing within the same capillary to ensure that DCU contents do not remain in the barrel. The number of such washing cycles and settings (vacuum and pulse duration) are defined by tapping the arrows for the settings above the blue **WASH** icon. **WASHES** value indicates the total number of cycles. **VAC, kPa** and **DUR, ms** preset vacuum strength and pulse duration, respectively. For example, the window on the side shows that six rinsing cycles of vacuum strength of 9kPa and duration of 3ms will be performed.

The progress of sample deposition will be shown in the **PLATE SETUP** window (see below) that may be accessed at any time by tapping the **PLATE SETUP** icon in the main side menu. Wells where collected samples are deposited will be indicated in green.

6.5 ADHESION

This window allows for the comparative estimation of single cell adhesion or detachment 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, “**TOTAL F, pN**”, and final force “**FINAL F, pN**”. Both values are based on the algorithm of the **RAMP** function. **TOTAL FORCE** is defined as the 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.

RAMP function represents a specific number (**PULSES**) of vacuum impulses starting with a predefined initial vacuum strength (**VAC, kPa**) raised by a predefined interval (**INTERVAL**) for each subsequent pulse.

Just like in **ACQUISITION** window, **VAC, kPa** and **DUR, ms** arrows at the top left provide controls for vacuum strength and duration used for the initial first pulse.



SELECT icon is used to select desired targets (cells or tissue regions). Selection is performed via computer screen and a mouse by pointing and clicking on a desired target. Multiple targets maybe selected for a single run.

The two icons below **SELECT** icon (**POOL** and **SINGLE**) are used to designate a specific collection-deposition mode. **POOL** icon is activated when multiple targets are selected and dispensed into a single tube.

Selecting the **SINGLE** icon activates the mode of collection in which each target is collected and deposited into a separate tube. This mode is used for single cell collection.

ABORT icon will interrupt the RAMP process at any time. Current values for TOTAL FORCE and FINAL FORCE will be given.

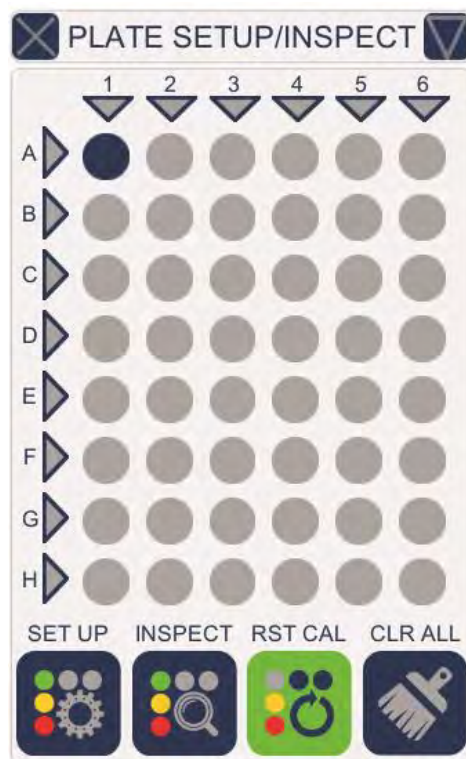
ACQ PLN icon indicates the current acquisition plane and may be used for its slight adjustment (recalibration) by bringing DCU to the current acquisition plane (e.g. 19000 μm) and then using the **CALIBRATION** window controls to adjust vertical position of the DCU tip.

6.6 PLATE SETUP/INSPECTION

The window represents 48 well universal sample holder used for sample collection and deposition with **A-pick™** automated system. Individual 0.1- and 0.2-ml PCR tubes, strips or 48-well plates are compatible with the holder. Samples may be collected from standard microscope glass slides or cell culture plates.

To set up a plate, move the motorized stage to position the center of the farthest back left well on the sample holder to align with your DCU tip position on the x-y plane. 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 grey when the stage position is calibrated, and the **SET UP** icon will turn green.

The collected samples will now be deposited from top left well to the bottom right well top to bottom per column, and from left column to right. Entire columns and rows may be selected by left-clicking on the corresponding triangle. A specific well may be prioritized by right-clicking on it once (prioritized first well will turn bright blue) or deselected by left-clicking (well will turn grey).



NOTE: When selecting individual wells, the samples will be deposited in the order in which the wells were selected.

INSPECT icon opens an additional window where before and after images for each collection. Wells with deposited samples appear red. Clicking on a red well will display the before and after image of the sample associated with it.

RST CAL icon clears the plate setup settings, allowing for re-calibration and **CLR ALL** icon clears all well selections along with all preselected targets associated with the wells.

6.7 SETTINGS

Left half of the screen allows for the selection (from left to right) of main light and calibration light intensities.

STBY DIST arrows permit setting desired stand by distance or distance of the DCU tip from the acquisition plane. The default distance for standby position during acquisition is 1 mm from the acquisition plane; however, this can be changed to a shorter distance on this page.

RET PLN arrows set the Retract plane (default setting is 0, i.e., the highest position). By lowering the vertical Retract position of the DCU, it may be quickly lowered or raised. Reducing this distance can minimize the time spent during calibration procedure or movement of DCU between different planes (e.g. between slide and test tubes).

Two **WHEEL DIRECTION** icons below offer a choice of direction of the calibration wheel to move DCU down: counterclockwise (**CCW**) or clockwise (**CW - default**).

The three **DCU SPEED** icons permit selection of DCU vertical movement speed when using the Z-AXIS arrows in CALIBRATION. The calibration wheel has a set speed of 1000 $\mu\text{m}/\text{sec}$ when turned quickly.

Location for the stored image, video, and log files created during the collection process is shown below. Tap the **Folder** icon to change destination.

EXIT PIKCELLS icon exits the program and the downward arrow at the bottom right opens another window where additional controls are located.

The downward arrow opens a dropdown menu for other controls, namely adding a crosshair to the view, measuring tool (Ruler icon), LOGO light control, and selection of camera.



6.7 CAMERA

A **CAMERA** window with standard image controls may be called out from the main menu to adjust image quality.

The camera controls directly access the camera that is connected to the control box.

7 Basic Operations

7.1 Initial Training Exercises with A-pick™

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

1. Start **A-pick™** using the power switch on the back panel of the Control box (green logo in the front will light up).
2. Initiate **PIKCELLS™** on your personal computer. Make sure that the correct camera is connected in order to run **A-pick™**. Under **SETTINGS**, tap on the downward arrow at the bottom right, and check that the system is connected to the correct camera. If not, tap on the X next to the camera listed and then choose the correct camera from the drop-down list, then tap on the play icon on the left. Here you can also turn on the grid for the screen. When finished, tap the downward arrow again to minimize the sub-window.



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

3. Place a marking on a histological glass slide and place it on the motorized stage with 2 drops of distilled water.
4. To attach the filter and DCU; rotate the Head 90° clockwise and attach a filter directly to the male luer connector on the **A-pick™** 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. The intensity may be adjusted using the sliding bar in the SETTINGS window.

5. Rotate **A-pick™** Head clockwise so that the DCU tip points down. Make sure that the head is turned all the way until it reaches a hard stop.
6. Before a run, the system requires calibration. Click on the **RET/SBY** icon to bring the DCU tip down by 15 mm. Using the green calibration wheel or **Z-AXIS** controls in the **CALIBRATION** window, carefully bring the DCU down another 2.0 to 3.0 mm. DCU position will be shown between **Z-AXIS** arrows, as well as at the bottom of the A-pick window.

NOTE: Single calibration wheel movements (one increment) or one click of a mouse wheel will move the capillary 1.5 μm per step. Moving the calibration wheel fast will increase the speed to 1000 $\mu\text{m}/\text{sec}$.

7. Switch to 4X objective and slowly moving it up locate the green DCU tip. When in focus, the annulus of the capillary tip appears as a bright green ring.
8. Use the linear stages/micromanipulators (**Figure 2**) to center the green annulus ring. It is recommended to locate the green annulus under the lowest magnification first.
9. Locate the marking on slide using low magnification objective and coarse and fine focus adjustment knobs to focus on marking.
10. Carefully bring the DCU down until the annulus of the capillary tip comes into focus as a bright green ring.
11. Stop lowering the DCU when the capillary tip moves with the glass slide when using the motorized stage. Make sure to move the slide/plate at the slowest speed using the joystick for the motorized stage or the hand icon 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 motorized stage will result in a movement of the capillary tip.
12. Click on the **RET/SBY** icon to bring the DCU to its highest *Starting* position and repeat the process from step 6.

7.2 DCU Calibration for Tissue Microdissection

Each newly attached 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 15-25 μm . For microdissection of brain anatomical regions (e.g. mouse hippocampus) ~50 μm ID DCU is recommended. Subanatomical regions, such as dentate gyrus or cortical layers, may be collected with ~30 μm ID DCUs. If fine microdissection is required, DCUs with <30 μm diameters should be used.



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

1. Start **A-pick™** using the power switch on the back panel of the Control box (green logo in the front will light up). Initiate **PIKCELLS™**. Place a tissue slide on the motorized 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 **A-pick™** Head 90° counterclockwise (**Figure 2**) to attach a filter directly to the male luer connector on the **A-pick™** Head. Then carefully handle a 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. The intensity may be adjusted using the sliding bar in the **SETTINGS** window.



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 **A-pick™** Head. When **A-pick™** is not in use, attach a new filter unit to prevent foreign objects from entering the system until next use.

4. Rotate **A-pick™** Head clockwise so that the DCU tip points down.
5. Using the green calibration wheel or **Z-AXIS** controls in the **CALIBRATION** window, carefully bring the DCU down until the capillary tip touches the surface of the liquid.

Optional: Click on the **RET/SBY** icon to bring the DCU tip down by 15 mm first before using the calibration wheel or **Z-AXIS** controls in the **CALIBRATION** window.

NOTE: Single calibration wheel movements (one increment) or one click of a computer mouse wheel will move the capillary 1.5 μm per step. Moving the calibration wheel fast will increase the speed to 1000 $\mu\text{m}/\text{sec}$.

6. Use the linear stages/micromanipulators to locate the DCU halo and position the halo adjacent to the tissue (**Figure 3A**). It is recommended to locate the green annulus under the lowest magnification.

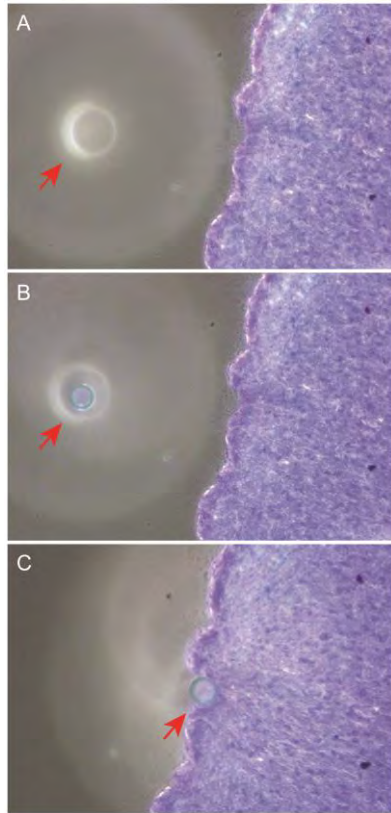


Figure 3: Three step procedure for DCU calibration. **A:** Initial finding of DCU tip (halo, shown with red arrow) in the field of vision; **B:** Lowering the DCU until the DCU tip and tissue section are both in focus; **C:** Moving the motorized stage to test if the DCU tip is in contact with the tissue 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 3B**). Lower the DCU until the capillary tip comes in contact with the tissue (**Figure 3C**). It is helpful to slowly move the microscope slide with the motorized 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 motorized 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 the green calibration wheel corresponds to 1.5 μm) until the motion of the motorized 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. Click on the **ACQ, μm** icon in the **CALIBRATION** window to set the calibrated acquisition plane/*Home* position. Use the **Z-AXIS** controls in the **CALIBRATION** window to lift the DCU slightly above the acquisition plane when moving the slide.
10. To re-calibrate, go to **ACQUISITION** window, and click on the **ACQ PLN** icon. This will bring the DCU down to the calibrated acquisition plane. Adjust the position of the DCU with the green calibration wheel or the **Z-AXIS** controls in the **CALIBRATION** window. Once the correct position is set, click on the **ACQ, μm** icon in the **CALIBRATION** window. This will set the new 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 and Tissue Collection** Section.

7.3 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 the cells with fresh medium to remove floating cells. Do not add new medium in excess, as the cells must be just barely covered.

1. Start **A-picK™** using the power switch on the back panel of the Control box once (green logo in the front will light up).
2. Place a cell plate/dish on the motorized stage.
3. Rotate the **A-picK™** Head 90° counterclockwise (**Figure 2**) to attach a filter and DCU. Attach a filter directly to the male luer connector on the **A-picK™** 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 **A-picK™** Head. When **A-picK™** is not in use, attach a new filter unit to prevent foreign objects from entering until next use.

4. Rotate **A-picK™** Head clockwise so that the DCU tip points down.
5. Using the green calibration wheel or **Z-AXIS** controls in the **CALIBRATION** window, carefully bring the DCU down until the capillary tip touches the surface of the liquid. **Optional:** If the system has not been calibrated yet, click on the **RET/SBY** icon to bring the DCU tip down by 15 mm before using the calibration wheel or **Z-AXIS** controls in the **CALIBRATION** window.

NOTE: Single calibration wheel movement (one increment) will move the DCU 1.5 μm per step. Moving the calibration wheel fast will increase the to 1000 $\mu\text{m}/\text{sec}$. Single click on the PLUS or the MINUS Z-AXIS controls will move the DCU 1.5 μm down or up, respectively.

6. Use the linear stages/micromanipulators (**Figure 2**) 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 slowly move the plate/dish with the motorized 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 3**).
8. When the DCU tip is in contact with the culture plate surface, a slight motion of the motorized 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 motorized 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 2**).



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. Click on the **ACQ, μm** icon to set the calibrated acquisition plane/ *Home* position. Lift the DCU before moving to different locations on the slide/plate.
11. To re-calibrate, go to **ACQUISITION** window and click on the **ACQ PLN** icon. This will bring the DCU down to the calibrated acquisition plane. Adjust the position of the DCU with the green calibration wheel or the **Z-AXIS** controls in the **CALIBRATION** window. Once the correct position is set, tap on the **ACQ, μm** icon in the **CALIBRATION** window. This will set the new acquisition plane. Use the **RET/SBY** icon to lift the DCU to the n the **ACQUISITION** window tap the **ACQ PLN** icon to bring the DCU back to the *Home* position. Re-position the capillary tip using the **Z-AXIS** controls in the **CALIBRATION** window or by rotating the calibration wheel and then push the calibration wheel again to set the new *Home* position.
12. You may re-calibrate at any time during the sampling procedure to designate a new acquisition plane.

13. Click on the **RET/SBY** icon and make sure that DCU is lifted high enough to clear the tops of tubes or any other objects on the motorized stage. Move the X-Y stage to the position where the sample needs to be dispensed.
14. Calibrate the DCU to identify dispense plane and set the *Home* position for the dispensing using the same sequence as described for the selection of acquisition plane.
15. Proceed to **Cell and Tissue Collection** Section



Caution: When the A-picK™ Head is rotated 90° counterclockwise, the DCU will not return to its initial *Starting* position. To return the DCU to the initial position with the RET/SBY icon. To avoid breaking the DCU tip we recommend returning a DCU to its initial *Starting* position before removing it from the unit.

7.4 Cell and Tissue Collection

Manual Mode

1. Place sample on the holder and then attach a DCU.
2. Open the software and allow it to initialize. The motorized stage will move to the back-left corner then to the front-right.
3. Tap on the SETTINGS icon and adjust the ring light and the green LED brightness.
4. Tap on the downward arrow at the bottom right, and check that the system is connected to the correct camera. If not, tap on the X next to the camera listed and then choose the correct camera from the drop-down list, then tap on the play icon on the left. Here you can also turn on the grid for the screen. When finished, tap the downward arrow again to minimize the sub-window.
5. Open the CALIBRATION window (top icon).
6. Move the stage to an area around where collection will take place and bring the sample in to focus.
7. Tap the RET/SBY to bring the DCU down by 15mm.
8. Using the linear stages at the base of the head unit, try to align the tip with the center of the objective.
9. Bring the DCU down to the plane of the samples to be collected. Use the up and down arrows (for faster movement – speed set under SETTINGS), Plus and Minus icons (for single step of 1.5 μm), or the wheel on the side of the control box (fast rotation for 1000 $\mu\text{m/s}$ and slow movement for single step). Note that the wheel direction can be changed in SETTINGS.
10. Once the DCU tip is in focus with the ROI, tap the ACQ icon to set the acquisition plane. If the DCU tip is off center on the screen, lift the DCU several steps up using the Minus icon, and use the linear stages at the base of the head to adjust its x-y position.

11. Preset dispense plane can be set by tapping one of the ACQ MODE icons. It is good practice to try and place the DCU tip in the center of the FOV/screen view. Using the grid is recommended.
12. ACQ and DISP planes may also be typed in manually, followed by Enter. The icons turn green when the planes are set.
13. Open the ACQUISITION and DISPENSE windows by tapping their icons (2nd and 3rd icons). Adjust the settings recommended for the application. At this time, also adjust STBY DIST under SETTINGS. This will set the distance the DCU lifts after each acquisition.
14. Move the stage to align the ROI with the DCU and tap GO! The DCU will come down and the vacuum will be applied, then it will lift to the designated standby position set under SETTINGS.
15. If pooling, move to the next ROI and repeat the previous step.
16. For dispensing, set the values desired under DISPENSE. Lift the DCU using RET/SBY icon or with the Z-AXIS up arrow and then move the stage so that the desired dispense location is in the center of view aligned with the DCU's x-y position.
17. Tap the DISPENSE icon. The DCU will come down to the designated dispense plane and pressure will be applied. After dispensing, the DCU lifts up to the Retract position designated under SETTINGS.

Manual Select Mode

1. Place sample on the holder and then attach a DCU.
2. Open the software and allow it to initialize. The motorized stage will move to the back-left corner then to the front-right.
3. Tap on the Settings icon and adjust the ring light and the green LED brightness.
4. Tap on the downward arrow at the bottom right, and check that the system is connected to the correct camera. If not, tap on the X next to the camera and choose the correct camera from the drop-down list, then tap on the play icon on the left. Here you can also turn on the grid for the screen. When finished, tap the downward arrow again to minimize the sub-window.
5. Open the CALIBRATION window (top icon).
6. Move the stage to an area around where collection will take place and bring the sample in to focus.
7. Tap the RET/SBY to bring the DCU down by 15mm.
8. Using the linear stages at the base of the head unit, try to align the tip with the center of the objective.
9. Bring the DCU down to the plane of the samples to be collected. Use the up and down arrows (for faster movement – speed set under SETTINGS), Plus and Minus icons (for

single step of 1.5 μm), or the wheel on the side of the control box (fast rotation for 1000 $\mu\text{m/s}$ and slow movement for single step). Note that the wheel direction can be changed in SETTINGS.

10. Once the DCU tip is in focus with the ROI, tap the ACQ icon to set the acquisition plane. If the DCU tip is off center on the screen, lift the DCU several steps up using the Minus icon, and use the linear stages at the base of the head to adjust its x-y position.
11. Preset dispense plane can be set by tapping one of the ACQ MODE icons. It is good practice to try and place the DCU tip in the center of the FOV/screen view. Using the grid is recommended.
12. ACQ and DISP planes may also be typed in manually, followed by Enter. The icons turn green when the planes are set.
13. If the plate holder will be used with plates or tubes, calibrate the well position by tapping on the PLATE SETUP icon (4th down from the top). Well A1 will be blinking.
14. Lift the DCU to the starting position (0) or high enough to clear the plate holder and anything that is mounted on the holder.
15. Move the stage so that the back-left most well is aligned with the center of the screen where the DCU is positioned. Tap on the blinking well. The SET UP icon at the bottom of the window will turn green. At a later time, this position can be recalibrated by tapping on the RST CAL (reset calibration), and then repeating the process.
16. Open the ACQUISITION and DISPENSE windows by tapping their icons (2nd and 3rd icons). Adjust the settings recommended for the application. At this time, also adjust STBY DIST under SETTINGS. This will set the distance the DCU lifts after each acquisition.
17. Move the stage so that there is a well contrasted view in the camera. In the CALIBRATION window, tap FIND XY (it will turn grey when activated) to calibrate the x-y plane.
18. If Auto Tip Positioning is desired, check the Auto Tip Pos box next to the FIND XY icon. The AUTO and TUNE icons will turn grey, and the DCU will come down to the acquisition plane.
19. The system will find the DCU tip automatically and provide its ID and OD. If adjustment is necessary, use the tuner that appears on the screen to make a better fit. After tuning, close the tuner by tapping on the X. This value will be used to calculate force. If the system is having a difficult time finding the tip, try increasing the green LED light intensity and lower the ring light intensity. If the Auto Tip Positioning is not checked, the system will assume that the DCU is in the center of the view and there will be no new measurement of the ID or the OD. It will assume the previously saved values for calculating force.
20. In the DISPENSE window, tap MANUAL (it will turn green when activated) to activate the manual selection mode.

21. In the ACQUISITION window tap SELECT (it will turn green when activated) to activate the select mode. A small bull's eye will allow selecting ROIs on the screen.
22. Make the selection(s) by clicking the ROI(s) with the bull's eye icon, then tap GO!. The system will automatically go to the ROI(s) and collect sample(s), then rest at standby. You can continue to select more ROI to continue collecting.
23. In the PLATE SETUP/INSPECT window, tap on INSPECT (it will turn green when activated).
24. Another window WELL INSPECT will appear to the right, providing images of before and after acquisition from the selected ROIs. The arrow at the bottom right allows the user to scroll multiple ROIs. The bottom left Go To icon (4 arrows) will move the stage to center that ROI in the field of view. The green circle to its right can be tapped to turn red if the user wishes to re-collect to that position. By tapping GO! again, the system will collect from that position again.
25. For dispensing, set the values desired under DISPENSE. Lift the DCU using RET/SBY icon or with the Z-AXIS up arrow and then move the stage so that the desired dispense location is in the center of view aligned with the DCU's x-y position. Tap the DISPENSE icon to dispense.
26. If the destination is one of the wells on the sample holder, left click with the mouse on the well in the PLATE SETUP/INSPECT window. The stage will automatically center the well in your view. Note: Make sure that the SET UP icon at the bottom left of the window is **not** selected (should be in dark blue). If the icon is in green, tap it once to deselect.
27. Tap the DISPENSE icon. The DCU will come down to the designated dispense plane and pressure will be applied. After dispensing, the DCU lifts up to the Retract position designated under SETTINGS.

Auto Dispense Mode

1. Place sample on the holder and then attach a DCU.
2. Open the software and allow it to initialize. The motorized stage will move to the back-left corner then to the front-right.
3. Tap on the SETTINGS icon and adjust the ring light and the green LED brightness.
4. Tap on the downward arrow at the bottom right, and check that the system is connected to the correct camera. If not, tap on the X next to the camera listed and then choose the correct camera from the drop-down list, then tap on the play icon on the left. Here you can also turn on the grid for the screen. When finished, tap the downward arrow again to minimize the sub-window.
5. Open the CALIBRATION window (top icon).

6. Move the stage to an area around where collection will take place and bring the sample in to focus.
7. Tap the RET/SBY to bring the DCU down by 15mm.
8. Using the linear stages at the base of the head unit, try to align the tip with the center of the objective.
9. Bring the DCU down to the plane of the samples to be collected. Use the up and down arrows (for faster movement – speed set under SETTINGS), Plus and Minus icons (for single step of 1.5 μm), or the wheel on the side of the control box (fast rotation for 1000 $\mu\text{m/s}$ and slow movement for single step). Note that the wheel direction can be changed in SETTINGS.
10. Once the DCU tip is in focus with the ROI, tap the ACQ icon to set the acquisition plane. If the DCU tip is off center on the screen, lift the DCU several steps up using the Minus icon, and use the linear stages at the base of the head to adjust its x-y position.
11. Preset dispense plane can be set by tapping one of the ACQ MODE icons. It is good practice to try and place the DCU tip in the center of the FOV/screen view. Using the grid is recommended.
12. ACQ and DISP planes may also be typed in manually, followed by Enter. The icons turn green when the planes are set.
13. Tap on the PLATE SETUP icon (4th down from the top). Well A1 will be blinking in the window.
14. Lift the DCU to the Starting position (0) or high enough to clear the plate holder and anything that is mounted on the holder.
15. Move the stage so that the back-left most well is aligned with the center of the screen where the DCU is positioned. Tap on the blinking well. The SET UP icon at the bottom of the window will turn green. At a later time, this position can be recalibrated by tapping on the RST CAL (reset calibration), and then repeating the process.
16. Open the ACQUISITION and DISPENSE windows by tapping their icons (2nd and 3rd icons). Adjust the settings recommended for the application. At this time, also adjust STBY DIST under SETTINGS. This will set the distance the DCU lifts after each acquisition.
17. Move the stage so that there is a well contrasted view in the camera. In the CALIBRATION window, tap FIND XY (it will turn grey when activated) to calibrate the x-y plane.
18. If Auto Tip Positioning is desired, check the Auto Tip Pos box next to the FIND XY icon. The AUTO and TUNE icons will turn grey, and the DCU will come down to the acquisition plane.
19. The system will find the DCU tip automatically and provide its ID and OD. If adjustment is necessary, use the tuner that appears on the screen to make a better fit. This value will be used to calculate force. If the system is having a difficult time finding the tip, try

increasing the green LED light intensity and lower the ring light intensity. If the Auto Tip Positioning is not checked, the system will assume that the DCU is in the center of the view and there will be no new measurement of the ID or the OD. It will assume the previously saved values for calculating force.

20. In the ACQUISITION window choose POOL or SINGLE (the activated mode will highlight in green), then tap SELECT (it will turn green when activated). A small bull's eye will allow selecting ROIs on the screen.
21. For dispensing, set the values desired in the DISPENSE window.
22. In PLATE SETUP/INSPECT window. The SET UP icon should be selected (in green). Select the wells for dispensing the collected samples by tapping on individual wells in the order they will be used. Entire rows or columns can be selected by tapping on the corresponding arrows. Default order is top to bottom, left to right.
23. Make the selection(s) by clicking the ROI(s) with the bull's eye icon, then tap GO!. The system will automatically go to the ROI(s) and collect sample(s), then rest at standby. You can continue to select more ROI to continue collecting. For POOL collection after one set of pooled ROIs are collected, the system will dispense in the first well, then return to the last acquisition location. Repeat the process for the subsequent wells.
24. In the PLATE SETUP/INSPECT window, tap on INSPECT (it will turn green when activated).
25. Another window WELL INSPECT will appear to the right, providing images of before and after acquisition from the selected ROIs. Left click on the well to inspect. For POOL mode, the arrow at the bottom right allows the user to scroll multiple ROIs. The bottom left Go To icon (4 arrows) will move the stage to center that ROI in the field of view.

7.5 Dispensing DCU Contents

1. Determine the plane for dispensing, and then in the **CALIBRATION** window, tap **DISP PLN** icon to set the Dispensing position.
2. In the **DISPENSE** window, set the pressure and duration parameters.
3. Go to the desired location for dispensing, and then tap **DISPENSE** icon. The DCU will move down to designated dispense plane and dispense its contents, and then move up the set Retract position.
4. If the DCU is not fully emptied, adjust the pressure and duration. Repeat the procedure.

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. To empty the contents from the DCU, rotate the A-picK™ Head 90° counterclockwise and carefully detach the DCU. Affix the DCU to the filter on the prepared syringe. 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 A-picK™ Head and re-calibrate to continue collection.

7.6 Moving Between Wells

1. Use the **RET/SBY** icon or **Z-AXIS** up arrow to raise the DCU to the *Starting* (0) position.
2. Move the plate using the motorized stage to position the next well under the field of view.
3. Use the **RET/SBY** icon, **Z-AXIS** down arrow, and or green calibration wheel to bring down the DCU to the desired acquisition plane.
4. Use the **ACQ PLN** icon in the **ACQUISITION** window to bring the DCU down to the most 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. Tap the **ACQ, μm** icon in the **CALIBRATION** window to set the new acquisition plane.
6. Resume collection

7.8 Changing DCUs

1. Use the **RET/SBY** icon or **Z-AXIS** up arrow to raise the DCU to the *Starting* (0) position.
2. Rotate the **A-picK™** Head 90° counterclockwise to access the DCU.
3. If necessary, using the **RET/SBY** icon or **Z-AXIS** down arrow, bring the DCU down/out for easier access.
4. Remove the DCU. Dispense the contents using a syringe if desired.
5. Attach the new DCU to the filter on the Head and use the **RET/SBY** icon or **Z-AXIS** up arrow to raise the DCU to the *Starting* (0) position.
6. Rotate the **A-picK™** Head clockwise so that the DCU tip points down.
7. Using the **RET/SBY** icon and/or **Z-AXIS** controls, bring the DCU down to the desired acquisition plane.
8. Align the annulus of the capillary tip to the center of the crosshairs using the linear stages/micromanipulators and/or adjust the z-axis position of the DCU using the green

calibration wheel or the PLUS/MINUS icons in the **CALIBRATION** window to determine the new *Home* position.

9. Tap the **ACQ, μm** icon I to designate the new acquisition plane/*Home* position.
10. Perform the same procedure if the Dispense plane needs to be set.
11. Resume collection.

7.9 After Use

1. Remove the DCU.
2. Replace the filter if it is suspected to be wet.
3. Use the **RET/SBY** icon, **Z-AXIS** up arrow or green calibration wheel to bring the DCU to the highest position (0).
4. Set the vacuum and pressure levels to 0 in the **ACQUISITION** and **DISPENSE** windows.
5. Turn off **A-picK™** using the power switch on the back of the control box and close PIKCELLS on the computer.

8 Sample Protocols

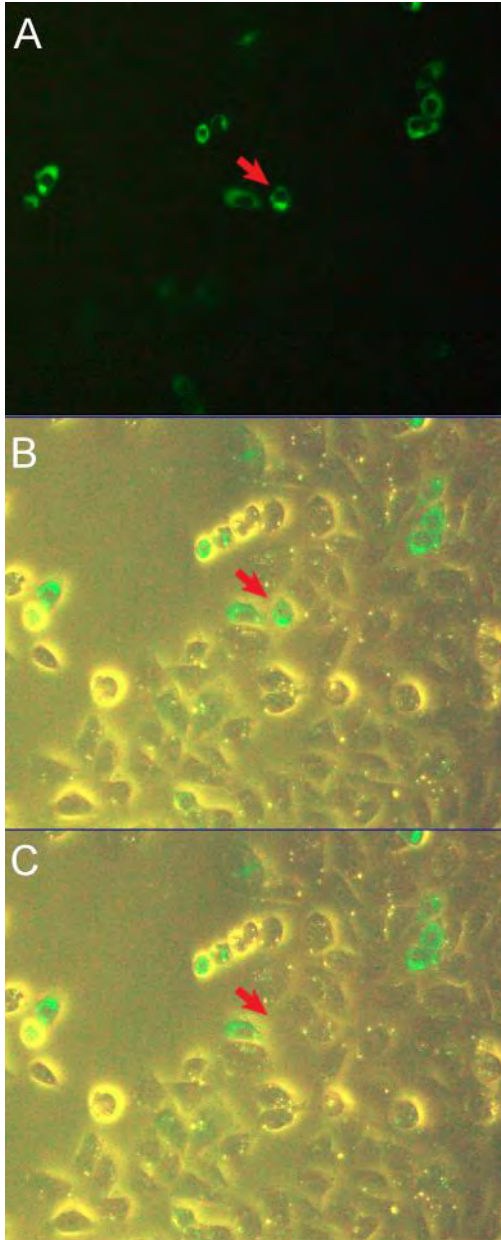
Protocol 1: Collection of Individual Adherent Cells from Culture Dishes

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 **A-picK™**. 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% CO₂ atmosphere.

Cell collection with A-picK™ 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 **DCU Calibration for Adherent Cell Cultures** and **Cell and Tissue Collection** sections. 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.

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.



When collecting fluorescently labeled cells (**Figure 4**), 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 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.

Figure 4: *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 may be 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 5**). 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:

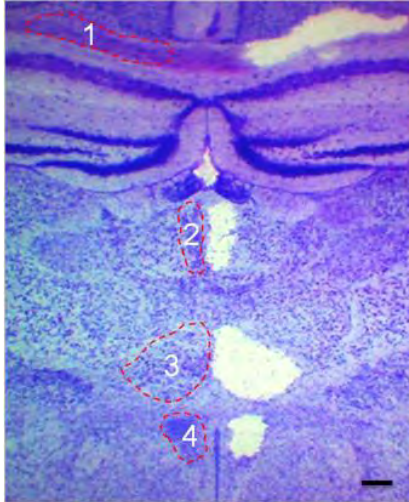
- Surgical instruments
- Standard animal perfusion apparatus and setup
- Cryostat
- Standard Phosphate Buffered Saline (PBS)
- Sucrose (15-20%) in PBS filtered
- 2-methylbutane
- Dry ice
- Glass microscope slides
- Cresyl violet, toluidine blue, hematoxylin, or any vital dyes
- Pipettor and sterile pipette tips
- **A-pick™** 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 **A-pick™**, 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 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 **A-pick™** Head. Follow instructions in **DCU Calibration for Tissue Microdissection** and **Cell and 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. $ID \leq 30 \mu m$ for single cell collection and $ID \approx 20-100 \mu m$ for subanatomical regions is recommended.

Figure 5. 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 $50 \mu m$. Scale bar = $250 \mu m$

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.

Manual Dispensing of Cell/Tissue from DCU:

Prior to removing a DCU from A-pick™ Head, prepare a syringe by pulling the syringe plunger back and attaching a filter to the syringe tip. Carefully remove the DCU (see section 7.8 Changing DCUs) 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 $-80^{\circ}C$.

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 **A-picK™** 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. **A-picK™** 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.ndx-instruments.com/support/downloads>

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 **A-picK™** 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 and 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>

Optional Manual Dispensing of Cell/Tissue from DCU:

9. Prior to removing a DCU from **A-picK™** Head, pull the syringe plunger back and attach a filter to the syringe tip.
10. Carefully remove the DCU from **A-picK™** 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 **A-pick™** 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 and 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>

Optional Manual Dispensing of Cell/Tissue from DCU:

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

9 Troubleshooting

If problems with **A-picK™** 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.
		Dispense into an empty tube to clear the clogging
		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 A-picK™ 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 calibration wheel.
3. DCU does not move	Calibration was set at minimum or maximum height.	Restart A-picK™ 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.

10 Technical Specifications

Specification	Description	
1. Illumination	Light source	120 LEDs ring light
2. Control Box Dimensions	410 mm (L) 289 mm (Control Box, W) x 112 mm (H) Allow min. 150mm for cabling at back in addition.	
3. Max Dimensions	18.8 in/478 mm (L) x 21.1 in/537 mm (W) plus 289 mm (Control Box, W) x 112 mm (H)	
4. Max Weight Approx.	10 kg not including adapter to microscope	
5. Pump	Max. Vacuum	70 kPa
	Max. Pressure	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	

11 System Performance

Description	Specifications
Resolution	Single Cell
Vacuum/pressure duration (Ts), seconds	0.1 s to 1.0 s
Vacuum strength, kPa	Max. 70 kPa
Pressure strength, kPa	Max. 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 \pm 1.5 cells/min
Mouse anterior horn motor neurons	12.0 \pm 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

12 Warranty and Liability

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SAFETY INFORMATION

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

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