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An Automated Mouse Tail Vascular Access System by Vision and Pressure Feedback

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Abstract

This paper develops an automated vascular access system (A-VAS) with novel vision-based vein and needle detection methods and real-time pressure feedback for murine drug delivery. Mouse tail vein injection is a routine but critical step for preclinical imaging applications. Due to the small vein diameter and external disturbances such as tail hair, pigmentation, and scales, identifying vein location is difficult and manual injections usually result in poor repeatability. To improve the injection accuracy, consistency, safety, and processing time, A-VAS was developed to overcome difficulties in vein detection noise rejection, robustness in needle tracking, and visual servoing integration with the mechatronics system.

Index Terms

Image processing; injection robot; marine vein detection; needle guidance

I. Introduction

Molecular imaging, such as computed tomography, magnetic resonance imaging, and positron emission tomography (PET), has become increasingly important for preclinical

models. Preclinical imaging like PET requires radiotracer to be injected into the target's venous system. The injection performance is measured by the amount of chemical material delivered to the target organ, which can be evaluated by chemical indicators [1], complete PET scans, or blood flashbacks [2]. For murine models, the most available vascular access is the tail vein. Because the diameter of a mouse tail vein is typically around 300 μm , a successful injection requires an accurate needle-vein alignment during insertion. Due to the small vein diameter and visual disturbances, quantitative research is often impeded by unstable drug delivery provided by manual injections [1]. To improve the injection yield rate, the literature shows that vein detection and insertion automation are the key components to providing researchers with repeatable vein access.

As a visual aid tool for technicians to perform manual injections, vein detection has been studied for many years for larger targets, such as blood sampling on human arms and hands. Near-infrared cameras are typically chosen for better skin penetration [3]. To reduce surface glare, these have been improved by optical polarization [4], [5]. In order to develop vein detection for mouse tail injections, it is worth noting that the lateral vein diameter of a lab mouse is approximately 10% of the size of the basilic vein in the human arm [6], [7]. In addition to the size difference, tail vein detection is disturbed by hair, scales, and pigmentation. Therefore, compared to human arms, automated injection for a mouse tail vein shows significantly greater challenges. Improved upon the image processing algorithms for human arm vein [8]–[12], an optimal image processing algorithm for mouse tail veins has been proposed [13].

By using the vein detection results, visual servoing allows full needle insertion automation to eliminate human-in-the-loop error. Earlier preclinical injection machines access larger targets, such as tumors [14], [15], or perform abdominal blood collection [16]. To access a target as small as the venous system of a small animal, a more maneuverable insertion robot with more degrees of freedom was developed to provide dexterous alignment and needle placement [17]. A semiautomated vascular access system (VAS) has been developed to provide visual vein detection information to the operator with motorized needle operation [18]. VAS performed comparably to a human operator. The most common failure mode was the error caused by tissue-needle interaction. Therefore, to perform the needle-vein alignment, a fully automated VAS is desired for murine drug delivery.

This paper demonstrates a compact automated vascular access system (A-VAS) for mouse tail injection that overcomes the real-time needle-vein alignment challenge by innovative mouse tail vein detection and robust needle registration algorithms. The device consists of a monitoring system and a multi-axial needle actuation system with graphical interface control. The full automation of A-VAS allows the device to avoid heuristic needle positioning calibration and automatically compensates for the needle-vein misalignment. To achieve the robust real-time needle-vein alignment, this study developed a predictive sum of square difference (SSD) method for needle registration and optimal cross-sectional reconstruction for vein detection. The remainder of this paper proceeds as follows: Section II demonstrates the mechanical design and configuration of the system; Sections III and IV describe the needle registration and tail vein detection method, respectively; Section VI illustrates the integration and performance of vision-based control and pressure feedback; Section V shows

the performance of A-VAS with experimental results. Concluding remarks are presented in Section VII

II. A-VAS Mechanical Design and System Setup

Murine drug delivery targets the most accessible vein in the mouse body. Schematic representation of a mouse tail cross section is shown in Fig. 1. Because lateral veins are the most readily available, they are commonly chosen for needle insertion. For manual injections, operators use their fingers to constrain the tail to align the needle with the speculated tail vein location. To provide visual assistance and insertion accuracy, VAS was designed to provide vein detection and actuated needle insertion [18]. As shown in Fig. 2, the design of the VAS curves the tail and provides constraints to the tip and base of the tail. Tail clamps were designed to secure the tail at the tip by two rubber wheels. By rotating the two clamping wheels, the tail can be pulled against the side of the holder to reduce movement during needle insertion. This mechanism is designed to secure the tail without causing damage or blocking blood circulation, which is similar to how technicians secure the tail with their hands while performing manual injections.

Because VAS requires the operator to manually control the insertion arm based on static vein detection, the major challenge comes from the needle-vein misalignment as the needle penetrates the tail skin and tissue. To automatically compensate for the needle-vein misalignment, A-VAS uses a monitoring system to navigate the needle actuation system. As seen in Fig. 2, the needle holder is driven by a four degree of freedom device that is actuated in the x , y , z , and θ direction by servo motors. The monitoring system consists of a dual camera system, a catheter pressure monitoring system, and a temperature regulation system. The dual camera system provides visual monitoring, vein detection, and needle registration. The catheter pressure monitoring system uses the syringe pressure to verify vein access. The temperature regulation system uses heating pads installed on the tail holder as well as the mouse bed to keep the vein dilated.

The system diagram of A-VAS is shown in Fig. 3. The host computer, a Dell Precision laptop, handles graphical user interface, image acquisition, image processing, trajectory generation, and data logging with Windows 7 operating system, 2.7-GHz dual-core CPU, and 8 Gb of RAM. The target machine, an Arduino mega 2560 microcontroller, acquires pressure reading from the pressure transducer and runs closed-loop x , y , and z motor control by encoder-based decoupled proportional-integral-derivative (PID) at 333 Hz with gains shown in Table I. The rotary stage is a CONEX-AGP closed-loop piezo actuator from Newport, which has an internal closed-loop controller and receives reference commands directly from the host computer. Motor y is a 10-mm stage from DynaOptic Motion, while motors x and z are DC motor XY stage 760X from Siskiyou. The pressure transducer is a Desert Medical 38-8000. Imaging Source DBK 41AU02.AS is used as the visible light camera that captures the tail and needle location in the z direction. The near-infrared camera, Imaging Source DMK 31BU03, is used to capture the near-infrared image of the tail in the x and y direction with linear polarization (Edmund Optics NT54-112) and near-infrared LED illumination (Fairchild QEC122). The dual-camera system is configured to provide 21.2, 21.2, and 72 $\mu\text{m}/\text{pixel}$ of resolution in x , y , and z , respectively.

The tracking performance of the actuation system is tested by a square-wave reference signal with 1000 μm of magnitude. The steady-state error in all three directions is less than 5 μm , while the overshoot is approximately 0.7%. The vision-based needle tracking performance accounts for needle deformation and interaction with the tail during the insertion process, providing 50–81 μm , 0–20 μm , and 8–64 μm of error in the lateral, axial, and depth direction, respectively.

A schematic view of the pressure sensor and syringe pump arrangement is shown in Fig. 4. The catheter is first filled with saline to maintain a stable pressure reading. Once the needle gains access to the vein, the transient increase in pressure commands A-VAS to halt at its current location in order to perform drug delivery.

III. Robust Needle Registration

In order to navigate the needle to approach the lateral tail vein, the camera system needs to identify the needle location and orientation. While gray-scale thresholding [19] is a common method for object recognition, its performance can be compromised by lighting condition variation and visual noise. To overcome this problem, the SSD method has been widely used for needle recognition in cell injections [20] for its robust tracking performance against target morphology.

As shown in Fig. 5, the SSD method finds the target movement of the region of interest (ROI) I_r by analyzing the difference between consecutive frames. It identifies the movement d by finding the ROI in the current frame, that is the most similar to the ROI in the previous frame

$$\begin{aligned} \Delta d &= (\Delta d_i, \Delta d_j) \\ &= \arg \min_{i,j} \sum_{i=0}^N \sum_{j=0}^N [I_r(i-N/2, j-N/2) - I_r^-]^2 \end{aligned}$$

where

$$\begin{aligned} I_r^- &= I_{t-\Delta t}(i_1:i_n, j_1:j_n) \\ I_r(i, j) &= I_t(i_1+i:i_n+i, j_1+j:j_n+j). \end{aligned} \quad (1)$$

Though SSD is able to handle target morphology, it is sensitive to rich background texture. For this application, SSD can be easily distracted by the surrounding tail features, such as scales, hair, and pigmentation. This makes the needle detection easily drift away from the correct needle location. To solve this problem, A-VAS restricts the SSD search range to the neighborhood predicted by the encoder reading from the actuation stage. Because d is determined within the intersected searching range, namely from h_1 to h_N in the horizontal direction and v_1 to v_N in the vertical direction, the tracking performance is greatly improved

$$\Delta d = \arg \min_{i,j} \sum_{i=h_1}^{h_N} \sum_{j=v_1}^{v_N} [I_r(i-N/2, j-N/2) - I_r^-]^2. \quad (2)$$

After identifying the gross needle location, the needle position and orientation in I_r need to be further determined. A lateral second-derivative filter D_r is applied to enhance the needle edge [14]. Applied to the edge-enhanced image, a least-square fit approach can determine the needle position and orientation by the slope m and offset b as shown in (3), in which $*$ denotes the convolution operator. An example of the needle tracking performance is shown in Fig. 6

$$\begin{aligned} (x_j, y_j) &= (\arg \max_i I_D(i=h_1:h_N, j), j) |_{j=v_1:v_N} \\ (m, b) &= \arg \min_{m,b} \sum_{j=v_1}^{v_N} (mx_j + b - y_j)^2 \end{aligned}$$

where

$$I_D = D_r * I_r, \quad D_r = \begin{bmatrix} 1 & -2 & 1 \end{bmatrix}. \quad (3)$$

For detection in the visible light camera, the needle is not overlaid with the tail. Therefore, the camera is able to recognize the needle location in the z direction by thresholding the ROI.

IV. Tail Vein Detection With Optimal Cross-sectional Reconstruction

Tail vein detection is achieved by applying image processing algorithms to the near-infrared image acquired by the vein detection system. As shown by the ROI in Fig. 7, large intensity change can be observed at the vein-tissue edge, and lateral tail vein can be recognized as the low-intensity area between the vein-tissue edges.

To improve the vein detection performance, high-frequency noise and low frequency bias are desired to be rejected. The intensity map of the acquired image within the ROI, denoted as I_i , is filtered by a two-dimensional Gaussian band-pass filter as shown in (4). For matrix symmetry, $i_{\min}$ and $j_{\min}$ are typically chosen to be $-i_{\max}$ and $-j_{\max}$, respectively

$$I_b = B_2 * (I_i - B_1 * I_i)$$

where

$$\begin{aligned} B_k(i+i_{\min}, j+j_{\min}) &= \frac{1}{2\pi\sigma_k^2} e^{-\frac{i^2+j^2}{2\sigma_k^2}} \\ i_{\min} &\leq i \leq i_{\max} \\ j_{\min} &\leq j \leq j_{\max}. \end{aligned} \quad (4)$$

A spatial low-pass filter B_1 is first applied to and then subtracted from the raw image. This operation serves as a high-pass filter that eliminates the low frequency bias. A secondary low-pass filter B_2 is then applied to eliminate the high-frequency noise. The combination of these operations functions as a spatial band-pass filter, where the bandwidth is determined by the parameters in B_1 and B_2 . Though this filter structure rejects both high frequency and low frequency disturbances, the formulation is computationally intensive and introduces latency to real-time feedback. To improve filter efficiency, the Gaussian filters B_1 and B_2 are combined into the form shown in (5). Note that I_1 has nonzero value only at the origin and the dimension is the same as filter B_1 . The benefit of this structure is that the filter can be precalculated. Because it requires only one convolution operation, this formulation is more suitable for real-time image processing calculation

$$I_b = (B_2 * (I_1 - B_1)) * I_i$$

where

$$I_1(i, j) = \begin{cases} 1, & i=0, j=0 \\ 0, & \text{otherwise.} \end{cases} \quad (5)$$

The standard deviation σ_1 and σ_2 for the band-pass filter are optimized based on the vein detection results. While the visual error is defined as the difference between the detection results and the reference location, the standard deviation S and the mean E of the visual error are formulated into a cost function to minimize on n_c images as shown in (6). By sweeping the μ value from 0 to 1, σ_1 from 20 to 140, and σ_2 from 2 to 8, the optimal σ_1 and σ_2 reside steadily around 75 and 4, respectively

$$C = \mu \sum_{i=1}^{n_c} E_i + (1 - \mu) \sum_{i=1}^{n_e} S_i. \quad (6)$$

The performance of this proposed band-pass filter is demonstrated in Fig. 8 with the parameters shown in Table II. The band-pass filter generates a smoother profile of the mouse tail, in which the low intensity value and high intensity change regions are recognized as the vein location and vein walls.

To identify the vein location, intuitively, gray-scale thresholding can be used to determine the coordinates of the vascular area and vein walls. However, as shown in Fig. 9, a suitable threshold value α varies between each cross section due to surface glare and lighting condition variation. Furthermore, the differences between mice, such as color, pigmentation, and hair, add additional difficulties to finding a robust and effective density threshold.

For each cross section, indicated by the zero-crossing point of the first-derivative of the profile, the vein location can be identified as the lowest intensity point. The vein-tissue edge location can also be found at the point of the largest intensity change, which can be located by the zero-crossing point of the second-derivative of the profile. The cross-sectional

derivative results are demonstrated in Fig. 10. For each image processing step, a selected cross section is displayed below the processed 2-D intensity map. It is shown that the numerical differentiation is sensitive to noise and introduces much false-positive detection.

To alleviate the effect of false-positive detection, this differentiation-based edge detection method requires a smoothing algorithm that is able to restore continuity while preserving the cross-sectional shape. This task can be treated as a data recovery problem: each cross section x is recovered from a corrupted cross-sectional measurement x_{cor} , and the recovered cross sections construct the smoothened ROI I_s

$$\begin{aligned} x_{\text{cor}} &= I_b(1:n, j) \\ I_s(1:n, j) &= x. \end{aligned} \quad (7)$$

A convex smoothing formulation is proposed to restore the shape of each cross section by improving its continuity without oversmoothing the image [13]

$$\begin{aligned} &\underset{x}{\text{minimize}} \|x - x_{\text{cor}}\|_2^2 + \mu\varphi \\ &\text{subject to } \varphi = \left(\sum_{i=1}^{n-1} |x_{i+1} - x_i|^m \right)^{1/m}. \end{aligned} \quad (8)$$

The first term of the objective function penalizes the difference between x and x_{cor} , and the second term constrains the signal continuity. The level of smoothing is determined by the weighting parameter μ : The higher the value is, the more smoothed the output is at the cost of deviating from the original signal. The proposed smoothing algorithm is applied to each cross section to eliminate the aforementioned false-positive zero-crossing detection. Since the proposed smoothing algorithm adopts the form of convex optimization with equality constraints, an explicit solution is available [21]. When m is chosen to be 2, it becomes a quadratic programming problem and the equality constraints can be incorporated into the objective function

$$\begin{aligned} \|x - x_{\text{cor}}\|_2^2 + \mu\varphi &= (x - x_{\text{cor}})^T (x - x_{\text{cor}}) + \mu x^T D^T D x \\ &= x^T (I + \mu D^T D) x - 2x_{\text{cor}}^T x + x_{\text{cor}}^T x_{\text{cor}} \end{aligned} \quad (9)$$

where

$$D = \underbrace{\begin{bmatrix} 1 & 0 & \cdots & 0 \\ -1 & 1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & \cdots & -1 & 1 \end{bmatrix}}_{n \times n}. \quad (10)$$

However, this scan-by-scan derivation is not computationally efficient and is, therefore, not suitable for real-time vision feedback. To improve solver efficiency, the optimization and reconstruction steps are combined into an equivalent matrix multiplication form. After

deriving the explicit solutions to all cross sections in the ROI, these solutions are used to reconstruct a smoothened intensity map for better vein detection performance

$$x^* = (I + \mu D^T D)^{-1} x_{\text{cor}} \quad (11)$$

$$I_s = I_b (I + \mu D^T D)^{-T}. \quad (12)$$

Fig. 11 shows a comparison between the cross-sectional Gaussian smoothing and the proposed continuity reconstruction filter. The proposed filter is shown to have better performance eliminating false-positive detection. Though more aggressive Gaussian filter can eliminate adequate amount of false-positive points, aggressive smoothing deviates the detection away from the true location.

The computational efficiency of the proposed vein detection method is verified on the host computer. When the ROI has a resolution of 435 by 101 pixels, this algorithm is able to update the vein detection result approximately every 200 ms, allowing for real-time tail movement monitoring.

V. Automated Insertion Control

The full automation of vascular access not only prevents human-in-the-loop mistakes, but also compensates for tail movement during insertion that typically happens to mice with short tails. Addressing the needle-tail interaction, the insertion process is actuated by a closed-loop controlled multi-axis stage with real-time vision-based reference governor and pressure monitoring. The control block diagram for the fully-automated insertion is shown in Fig. 12. A-VAS is initiated by the needle homing module. After the needle tip is homed at the home position, the vein insertion module generates reference command based on the needle and vein location and the syringe pressure feedback.

To make the needle's ROI I_r invariant to needle replacement and adjustment, the homing process needs to rest the needle tip at a predefined home location. Because a limit switch is unable to compensate for the needle length and clamping condition variation, a vision-based homing scheme is developed. The homing process is shown in Fig. 13, where the stage moves laterally in the x direction for background calibration and then performs vision-based homing. The needle tip location can be determined by subtracting the acquired image [see Fig. 13(c)] from the calibration image [see Fig. 13(b)]. This approach is advantageous against lighting changes and needle variation.

After the homing process is finished, the needle is desired to be aligned with the tail vein to perform insertion. The needle and vein are considered to be aligned if the absolute value of the alignment error e_x is less than e_{max} , where e_{max} is determined by the target vein diameter. This alignment condition is used to switch between the alignment mode and the insertion mode as shown in (13). The automated needle insertion is initiated by aligning the needle with the detected vein location with gain K_x . Once the alignment is achieved, the needle moves forward in the y direction with velocity gain v_z . If the alignment error e_x

exceeds $e_{\max}$ during insertion, the system would reenter the alignment mode and the forward motion would pause until the alignment error converges. This mechanism allows A-VAS to compensate for the misalignment caused by the interaction between the inserting needle and tail tissues. From Fig. 14, the needle tracking algorithm is shown to follow the needle movement, while the needle is aligning with the detected vein location

$$\begin{bmatrix} r_x(t+\Delta t) \\ r_y(t+\Delta t) \\ r_z(t+\Delta t) \end{bmatrix} = \begin{cases} \begin{bmatrix} r_x(t) + K_x e_x \Delta t \\ r_y(t) \\ r_z(t) \end{bmatrix}, & \text{if } |e_x| \geq e_{\max} \\ \begin{bmatrix} r_x(t) \\ r_y(t) \\ r_z(t) + v_z \Delta t \end{bmatrix}, & \text{otherwise} \end{cases} \quad (13)$$

As the needle moves forward, the transient change in pressure transducer reading is used as an indicator to verify vein access. When a transient pressure change is detected, the insertion motion will be halted for radiotracer injection as shown in Fig. 15. Fig. 16 shows the infrared and visible light camera views when initiating, during and finishing the insertion. Fig. 17 demonstrates the blood flashback after the needle is pulled out. The blood flashback is a visual verification of the needle being successfully inserted into the tail vein along with large pressure rise.

VI. Automated Insertion Results

To provide adequate base for experimental verification, murine vascularization characterization [6] and surgical injection study [23] typically choose 5–6 mice exclusively to verify experimental results. C57 mice are commonly selected for vein insertion experiments for their high level of consistency at UCLA and other research facilities [22]–[24]. Trials of the A-VAS system were conducted on 11 C57 black mice aged 2–9 months old, used for experiments with the A-VAS system without sharing with other laboratory experiments. Black mice were chosen for the most challenging tail hair and scales disturbances. Experimental procedure is the same as VAS [18]: mice are given 200- μ L subcutaneous saline 30 min before the experiment to increase the blood pressure and to prevent from dehydration, and are anesthetized by a mixture of vaporized isoflurane-oxygen 5 to 10 minutes before the experiment.

For these tests, a successful injection on any given mouse is defined as registering a 31.3 mmHg rise in catheter pressure accompanying the motor stop condition and visual verification of blood flashback. Test results are shown in Table III. As shown in this table, the system has an 82% (9/11) yield rate, which is higher than 80% (4/5) of the semiautomated VAS [18] with larger test base and 60% (18/30) of manual injections [1].

VII. Conclusion

To provide stable vein access for preclinical study on murine models, an automated vascular access system (A-VAS) has been developed to overcome the vein recognition difficulty and avoid human-in-the-loop error. Repeatable vein access is provided by temperature

regulation, tail constraints, needle-vein detection, and catheter pressure monitoring. By identifying the tail vein location, needle position and orientation, the needle is able to drive automatically toward the identified mouse tail vein with real-time alignment correction. While manual injections provide vascular access for 60% of insertions, experimental results proves that A-VAS can provide vein access with an 82% yield rate by fully automated insertion.

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Fig. 1.
Mouse tail cross section with blood vessel locations.

Fig. 2.
Mechanical Design of the VAS.

Fig. 3.
System Diagram of the VAS.

Fig. 4.
Catheter pressure sensing.

Fig. 5.
SSD method used in needle tracking.

Fig. 6.
Needle Recognition in ROI.

Fig. 7.
Tail image acquired by the near-infrared camera.

Fig. 8. Near-infrared tail image of the ROI. (a) Row image. (b) Bandpass filtered image.

Fig. 9.

Thresholding on the near-infrared tail vein image in the ROI in Fig. 7. (a) Raw. (b) Filtered. (c) $\alpha = 0.5$. (d) $\alpha = 0.55$. (e) $\alpha = 0.60$. (f) $\alpha = 0.65$.

Fig. 10.

Vein detection image processing. (a) Raw. (b) Bandpass. (c) $\frac{d}{dx}$. (d) $\frac{d^2}{dx^2}$.

Fig. 11.
Smoothing method comparison for vein detection. (a) Gaussian. (b) Convex.

Fig. 12.
Block diagram of needle tip homing and vein insertion control.

Fig. 13.
Homing process. (a) Initialization. (b) Calibration. (c) Homing.

Fig. 14. Alignment Process: Encoder readings (-), vision (○), and alignment errors (-.-).

Fig. 15.
Pressure reading and insertion control. (a) Vein accessible. (b) Vein inaccessible.

Fig. 16.

Top and side view of the automated insertion process. (a) Initialize alignment, $e_x = 3500 \mu\text{m}$.

(b) During alignment, $e_x = 1000 \mu\text{m}$. (c) Alignment complete, $e_x = 257 \mu\text{m}$. (d) During

insertion, $e_x = 200 \mu\text{m}$. (e) Insertion complete, $e_x = 76 \mu\text{m}$.

Fig. 17.
Blood flashback for a successful insertion. (a) Successful Injection. (b) Zoomed-in view.

TABLE I

PID Gains

Motor	K_p	K_i	K_d
x	15	5	1
y	25	10	2
z	15	5	1

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TABLE II

Filter Parameters

Filter	σ	$i_{\min}$	$i_{\max}$	$j_{\min}$	$j_{\max}$
B_1	75	-30	30	-30	30
B_2	4	10	-10	10	-10

TABLE III**A-VAS Injection Results**

C57 Mouse	Pressure Change [mmHg]	Blood Flashback	Success
1	950	Yes	Yes
2	280	Yes	Yes
3	35	No	No
4	840	Yes	Yes
5	110	Yes	Yes
6	164	Yes	Yes
7	197	Yes	Yes
8	544	Yes	Yes
9	190	Yes	Yes
10	711	Yes	Yes
11	10	No	No